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ASSISTED BY

D. KEILIN, Sc.D.

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VOLUME XVIII SEPTEMBER, 1926 No. 3

A SUPPLEMENTARY NOTE ON *MONOPYLIDIUM CHANDLERI* AND OTHER RELATED SPECIES.

BY M. A. MOGHE, M.A. M.Sc.

Hislop College, Nagpur, C.P., India.

IN 1925 I described a new species of *Monopylidium*, *M. chandleri*[1], proposed a new subgenus *Macracanthus* for this and two related species, and proposed a new genus *Southwellia* for the species *Monopylidium gallinarum* Southwell 1921. Dr M. C. Hall has very kindly drawn my attention to certain errors and omissions in this paper which are herein corrected.

The subgeneric name *Macracanthus* which I proposed to include *M. chandleri* Moghe 1925, *M. stercorarium* Baylis 1919 and *M. macracanthus* Fuhr. 1908, is preoccupied by Chaudoir, 1846. I therefore propose the name *Megalacanthus* as a subgeneric name, and designate *Monopylidium* (*Megalacanthus*) *chandleri* as the type species. I have not had an opportunity to examine specimens of *Monopylidium stercorarium* and *M. macracanthus*, but it is evident from the published descriptions that they also belong to the subgenus *Megalacanthus*.

Meggitt (1926, p. 233)[2], in a footnote, remarks that the creation of a separate subgenus on the sole basis of rostellar hooks does not appear justifiable. In my opinion, the creation of subgenera in a large and unwieldy genus is largely a matter of convenience; this is, in fact, the principal aim of all classification. If there were every gradation within the genus in number and size of hooks, we should not be justified in making any arbitrary division, but when one group of species differs distinctly from another, the degree of difference being considerable, it is not improbable that a true genetic relationship is indicated. Even if this is not so, unless it is clear that the species included in one group are not more closely related to each other than to any members of the other group, the matter of convenience of reference should have considerable weight when the creation of subgenera, though not necessarily of genera, is contemplated. A subgenus merely designates a group of species within a genus, grouped because they have certain characters in common which are not shared by other species. There would be no advantage in recognising group differences in a genus containing few species, whilst the contrary may hold

[1] Moghe, M. A. (X. 1925). A new species of *Monopylidium*, *M. chandleri* from the Red Nettled Lapwing (*Sarcogrammus indicus* Stolickza), with a key to the species of *Monopylidium*, *Parasitology*, XVII. 395–400.

[2] Meggitt, F. J. (1926). The Tapeworms of the Domestic Fowl, *J. Burma-Res. Soc.* XV. 222–243.

with a genus comprising many species that may conveniently be grouped in subgenera, this leading to simplification.

I proposed the name *Southwellia* for a new subgenus to include *Monopylidium gallinarum* Southwell 1921, which therefore becomes *Southwellia gallinarum* (Southwell 1921); this species is designated as the type of the genus. So far as I can judge from the literature, there are no other species which can definitely be assigned to this genus, though, as remarked in my earlier paper, *M. rostellata* Fuhr. 1908 from *Himantopus mexicanus*, in which the hooks are lost, but the genital pores are described as unilateral, may also be found to be a species of *Southwellia*.

Meggitt (1926, p. 233) independently suggested the same name for this species. His paper went to press before mine, but was published at a later date, consequently *Southwellia* Meggitt becomes a synonym of *Southwellia* Moghe. Meggitt erroneously changes *gallinarum* to *gallinaria*, presumably to make it agree in gender with the new genus name, but, since *gallinarum* is a plural genitive, it requires no modification, *gallinaria*, therefore, also falls into synonymy.

I am thankful to Dr Hall for his kindness in drawing my attention to the matters which are here cleared up.

(*MS. received for publication* 19. IV. 1926.—Ed.)

THE HERPYLLOBIIDAE.

A FAMILY OF COPEPODA PARASITIC ON POLYNOID WORMS.

By W. HAROLD LEIGH-SHARPE, M.Sc. (Lond.).

(With 11 Text-figures.)

Family **HERPYLLOBIIDAE.**

Family Diagnosis. Copepoda parasitic dorsally on Polynoid Worms.

Female with body divided into cephalothorax (head and neck) and trunk. No appendages articulated or otherwise on head or neck. No traces of segmentation. No mouth nor alimentary canal. Cuticle thin. Head and neck buried in the tissues of the host; the head converted into a voluminous organ whose only function is to absorb nutritive materials by endosmosis through expansions or rootlets. Head and neck at right angles to the middle of the trunk. A chitinous ring is usually present at the base of the neck at its insertion on the trunk. Genital apertures wide, the pyriform eggstrings issuing therefrom.

Male does not exhibit much degeneration.

The foregoing diagnosis excludes the genera *Saccopsis*, *Bradophila*, *Oestrella* which were included in this family by Hansen (1892). The doubtful genus *Trophoniphila* McIntosh (1885) might be included but is insufficiently described for purposes of classification. *Selioides bolbroei* Levinsen (1877–8, Pl. VI, figs. 5–11), although occurring on a Polynoid worm, *Harmothoë imbricata*, off Greenland, has articulated appendages and is therefore not included in this family.

In the erection of genera the following female characters have been relied on: (1) appearance of the head and its expansions, (2) length and curvature of neck, (3) presence or absence of chitinous ring, (4) shape of trunk and presence or absence of appendages thereon.

Genus I. **HERPYLLOBIUS** Steenstrup and Lütken 1861.

Head expanded and flattened with crenated edges, concave anteriorly. *Neck* somewhat short, straight, extremely slender. Chitinous ring absent. *Trunk* spherical, without appendages.

One species: *H. arcticus* and a doubtful species *H. affinis*.

H. arcticus Steenstrup and Lütken 1861.

Host: *Lepidonotus* Sp.

Locality: Greenland.

The sole known species of the genus. Almost all the authors cited under References at the end of this paper have recorded and figured an animal which they call *Herpyllobius arcticus* Steenstrup and Lütken. No two of these authors agree as to the conformation of the head; most of them have concluded that the genera *Herpyllobius* and *Silenium* are synonymous, which is not my opinion. Moreover, Steenstrup and Lütken in 1869 regarded them as distinct because they refigured both genera. (Fig. 1 is taken from their figure of *H. arcticus*.) Among other hosts upon which the alleged *H. arcticus* has been found are *Eunoa oerstedi* and *Harmothoë imbricata*. It does not follow that all of these copepods are of one species, indeed I consider that most of them belong in the genus *Silenium* and some even in the genus *Rhizorhina*.

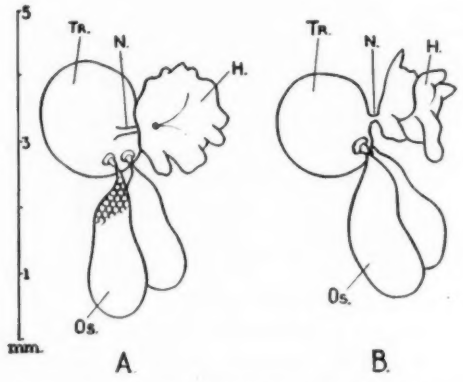

Fig. 1. *Herpyllobius arcticus* ♀. *A.* in postero-lateral aspect, *B.* in lateral aspect. *H.* = head; *N.* = neck; *Tr.* = trunk; *Os.* = ovisacs. (After Steenstrup and Lütken.)

H. affinis Hansen 1887.

(*Doubtful species.*)

Host: *Harmothoë badia*.

The original description renders the determination of the genus impossible. The body-form and the conformation of the head resemble that of *Hedyphanella*, the latter having more root-like expansions than in *Hedyphanella* and approximating to *Rhizorhina*. The absence of the chitinous neck-ring is the only justification for the inclusion of this species under *Herpyllobius*. The ovisacs are elongated and do not resemble the form typical of this family.

Genus II. **SILENIUM** Krøyer 1863.

Head unknown. *Neck* short, straight, stout, chitinous ring present. *Trunk* ovoid, somewhat pointed anteriorly. A posterior enlargement from which both ovisacs issue. One species: *S. polynoës*.

S. polynoës Krøyer 1863.

HOST: *Polynoë cirrata* and *P. scabra*.

LOCALITY: Greenland and Norway.

It is obvious from Krøyer's original figure, herein reproduced (Fig. 2), that he did not dissect out the head, but he demonstrated the presence of the chitinous ring.

Genus III. **HEDYPHANELLA** n.g.

SYN. *Herpyllobius arcticus* Brian 1914.

Head clavate, with fleshy expansions resembling the fungus *Morchella*. *Neck* short, straight, stout. Chitinous ring present. *Trunk* ovoid, laterally compressed, without appendages.

One species: *H. superba*.

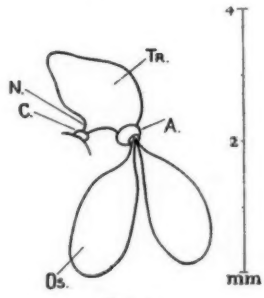

Fig. 2.

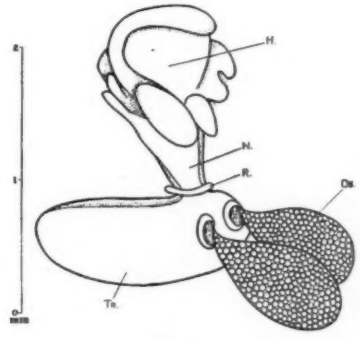

Fig. 3.

Fig. 2. *Silenium polynoës* ♀. *C.* = chitinous ring; *N.* = neck; *Tr.* = Trunk; *A.* = posterior enlargement; *Os.* = ovisacs. (After Krøyer.)

Fig. 3. *Hedyphanella superba* ♀. *H.* = head; *N.* = neck; *R.* = chitinous ring; *Tr.* = trunk; *Os.* = ovisacs. (After Brian.)

H. superba n.sp.

HOST: *Gattyana* [*Nychia*] *cirrosa* (Pallas).

LOCALITY: Terra Nova (Brian); Plymouth (Leigh-Sharpe).

First described by Brian (1914), without being named. He unfortunately placed it in the genus *Herpyllobius*, whereas it very closely resembles *Silenium*. His excellent original illustration is herein reproduced (Fig. 3).

Genus IV. **SARSILENIUM** n.g.

SYN. *Silenium*. M. Sars (1870).

Head unknown. *Neck* stout. Chitinous ring present. *Trunk* spherical. Abdominal appendages present in the form of two spherical lobes.

One species: *S. crassirostris*.

S. crassirostris (M. Sars 1870).

HOST: *Evarne impar* (Johnston).

LOCALITY: Drøbak Fjord; Plymouth (C. D. Vallence's collection).

The species is illustrated in Fig. 4 (♀) and Fig. 5 (♂). It is curious that only immature females, without ovisacs, were collected. The females bore males.

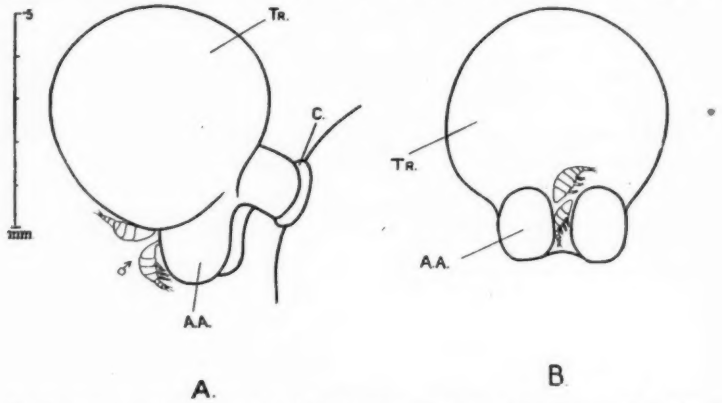

Fig. 4. *Sarsilenium crassirostris* ♀. *A.* in lateral aspect, *B.* in posterior aspect. *C.* = chitinous ring; *Tr.* = trunk; *A.A.* = abdominal appendages; ♂ = males. (After M. Sars.)

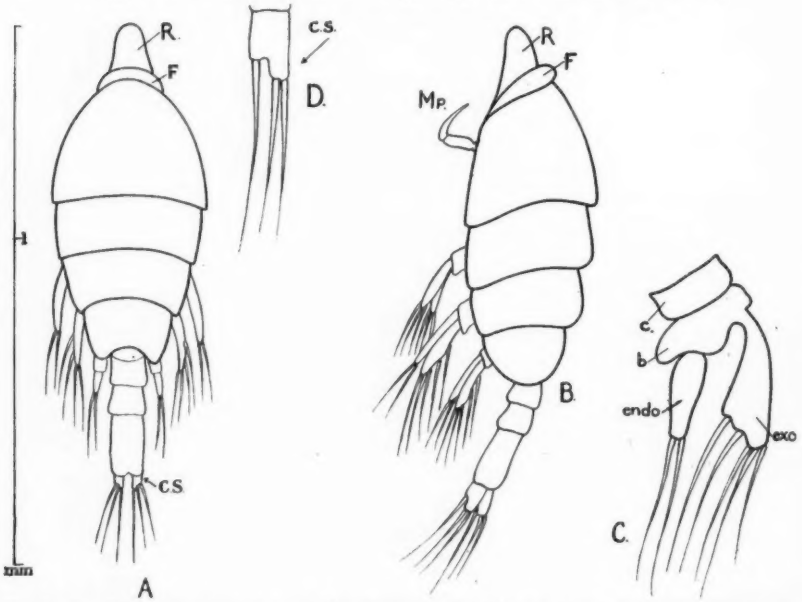

Fig. 5. *Sarsilenium crassirostris* ♂. *A.* dorsal aspect; *B.* lateral aspect; *C.* a thoracic appendage; *D.* one caudal style enlarged. *R.* = rostrum; *F.* = fold; *Mp.* = maxillipede; *C.S.* = caudal styles; *c.* = coxopodite; *b.* = basipodite; *endo.* = endopodite; *exo.* = exopodite. (After M. Sars.)

Genus V. **EURYSILENIUM** M. Sars 1870.

Head and *Neck* unknown. Apparently no chitinous ring. *Trunk* somewhat spherical with two posterior expansions and a small median abdominal appendage. Ovisacs spherical when young; as in Fig. 6 in older specimens.

One species: *E. truncatum* and one doubtful species, *E. oblongum*.

E. truncatum M. Sars 1870.

HOST: *Harmothoë imbricata* (Linn.).

LOCALITY: Drøbak Fjord.

The species is illustrated in Fig. 6. Though the type-host is common at Plymouth, I have not yet found the copepod upon it.

E. oblongum Hansen 1887.

(*Doubtful genus and species.*)

HOST: *Harmothoë badia*.

Trunk oblong, of relatively large size, ovisacs curled. Genus undeterminable. The author's illustration is herein reproduced (Fig. 7) without further comment.

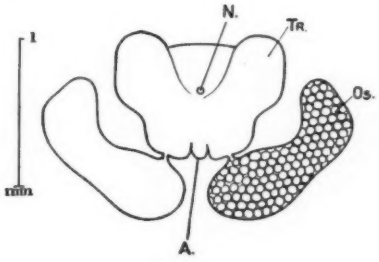

Fig. 6.

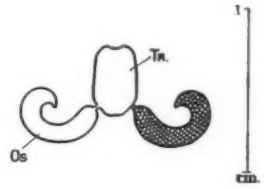

Fig. 7.

Fig. 6. *Eurysilenium truncatum* ♀. *N.* = point where neck was broken off; *Tr.* = posterior expansions of trunk; *A.* = abdominal appendage; *Os.* = ovisacs. (After M. Sars.)

Fig. 7. *Eurysilenium oblongum* ♀. *Tr.* = trunk; *Os.* = ovisacs. (After Hansen.)

Genus VI. **RHIZORHINA** Hansen 1892.

Head rhizoid. *Neck* short, slender, not distinguishable from the head save for the absence of rootlets. Chitinous ring absent. *Trunk* napiform, without appendages.

One species: *R. ampeliscae*.

R. ampeliscae Hansen 1892.

HOST: *Ampelisca laevigata*.

LOCALITY: Denmark and Norway.

Although not occurring on a Polynoid worm but upon an Amphipod, it seems to me that the species (Fig. 8) may well be included in this family, although the neck arises at the apex instead of midway along the trunk as described under the family diagnosis. The neck forms a right angle with the trunk.

Genus VII. **PHALLUSIELLA** n.g.

Head phalloid. *Neck* slender, curved, elongated. Chitinous ring present. *Trunk* enlarged anteriorly, narrowest in the middle, bilobed posteriorly forming sub-spherical appendages.

Two species: *P. psalliota*, *P. vera*.

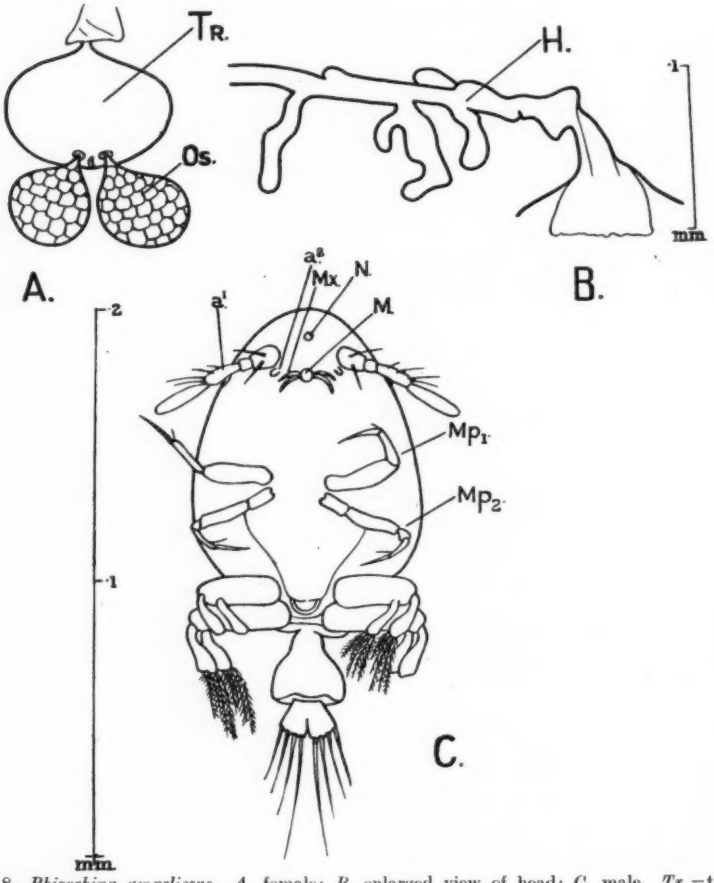

Fig. 8. *Rhizorhina ampeliscae*. *A*. female; *B*. enlarged view of head; *C*. male. *Tr.* = trunk; *Os.* = ovisacs. *H.* = head; a^1. = antennule; a^2. = antenna; *N.* = neck; *M.* = mouth; *Mx.* = maxilla; Mp_1. = first maxillipede; Mp_2. = second maxillipede. (After Hansen.)

P. psalliota n.sp.

Host: *Lagisca floccosa* (Savigny).

Locality: Plymouth.

Head smooth, concave posteriorly, resembling the pileus of the mushroom *Psalliota*. Anteriorly, a slight depression which may be the vestige of a stomodaeum (Fig. 9, *S*.). *Neck* stouter, and not so curved as in *P. vera*.

P. vera n.sp.

HOST: *Malmgrenia castanea* McIntosh.

LOCALITY: Looe-Eddystone grounds, Plymouth, 1. vi. 1914.

Head with fleshy expansions, no concavity. *Neck* long, slender, more curved than in *P. psalliota*. *Trunk* very narrow in the middle. The species is illustrated in Fig. 10.

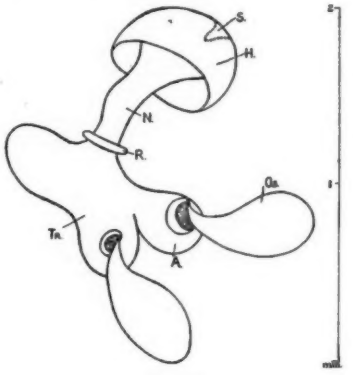

Fig. 9.

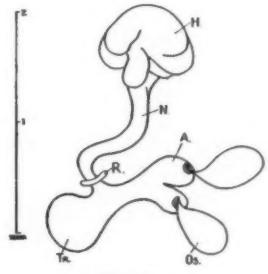

Fig. 10.

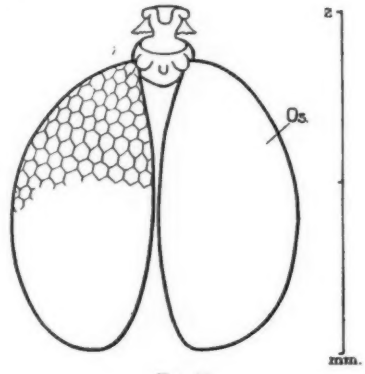

Fig. 11.

Fig. 9. *Phallusiella psalliota* ♀. *H.* = head; *N.* = neck; *S.* = ? stomodaeum; *R.* = chitinous ring; *Tr.* = trunk; *A.* abdominal appendage; *Os.* = ovisacs.

Fig. 10. *Phallusiella vera* ♀. *H.* = head; *N.* = neck; *R.* = chitinous ring; *Tr.* = trunk; *A.* = abdominal appendage; *Os.* = ovisacs.

Fig. 11. *Bradophila pygmaea* ♀. *Os.* = ovisacs. (After Levinsen.)

APPENDIX.

I here include an illustration (Fig. 11) of *Bradophila pygmaea* Levinsen (1877–8), outlined after the author's figure. It is parasitic on *Brada*. Although I do not regard the species as belonging to the family HERPYLLOBIIDAE, yet

it exhibits a number of characters that indicate relationship. The head and ovisacs alone resemble those of the HERPYLLOBIIDAE. The head has symmetrically arranged fleshy excrescences with two lateral serrate horns. The ovisacs are enormous relatively to the body. The neck is very short and there is no chitinous ring. The trunk is napiform, and there are two spherical abdominal processes (from which the ovisacs issue?) as in *Phallusiella*, and a small medium process as in *Eurysilenium*. The principal reason for excluding it from the HERPYLLOBIIDAE is that the head and neck are not perpendicular to the middle of the trunk.

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(*MS. received for publication* 28. IV. 1926.—Ed.)

RECHERCHES PHYSICO-CHIMIQUES SUR LES LIQUIDES DE PARASITES.

(*ASCARIS*) *II.*

PAR WILLIAM H. SCHOPFER,

Assistant à l'Université de Genève.

(*Laboratoire de Parasitologie, Université de Genève.*)

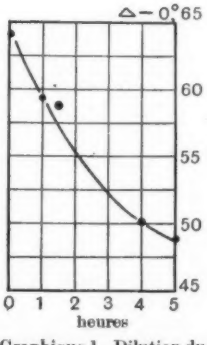
(Avec 5 Graphiques.)

DANS un travail publié précédemment dans cette revue, nous avons montré que la concentration moléculaire du liquide cœlomique d'*Ascaris megalocephala* était voisine de $\Delta = -0°, 63$ avec de petites variations; d'après nos expériences, l'*Ascaris* était susceptible d'une régulation osmotique; il augmente de poids dans une solution hypotonique par rapport à son liquide; il y a pénétration d'eau; dans une solution hypertonique il diminue de poids (départ d'eau).

La marche de la dilution du liquide cœlomique lorsque l'animal est plongé dans une solution hypotonique est la suivante:

Après	Δ
0 h. 0 m.	− 0°, 645
1 h.	− 0°, 586
1 h. 30 m.	− 0°, 576
4 h.	− 0°, 504
5 h.	− 0°, 480.

Nous avons bien insisté sur le fait que ces phénomènes se produisaient dans des solutions de concentration extrême; que les résultats étaient valables *in-vitro* et hors des conditions physiologiques. Une critique peut en effet être faite; lorsqu'on opère avec des animaux marins il est facile de modifier le milieu extérieur, l'eau de mer, en le diluant ou en l'évaporant sans rien changer à sa composition qualitative. Par contre, lorsque je place dans de l'eau courante, de l'eau distillée, dans une solution de NaCl concentrée des *Ascaris* dont le milieu normal est le liquide intestinal, je suis hors des conditions physiologiques, et mes solutions n'ont qualitativement rien de commun avec le milieu normal de l'animal.

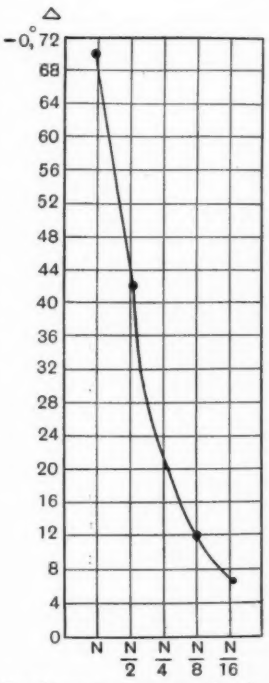


Graphique 1. Dilution du liquide en fonction du séjour dans une solution hypotonique (eau distillée).

Les phénomènes osmotiques présentés par les membranes au contact de l'eau distillée (qui effectivement est un mauvais liquide d'expérience), peuvent ne rien avoir de commun avec ceux que présentent les membranes au contact de leur milieu normal; elles peuvent être altérées et les résultats obtenus ne peuvent pas être généralisés. Il est certain que la très forte endosmose qui se produit lorsque l'animal est plongé dans l'eau courante, n'a jamais l'occasion de se manifester dans les conditions physiologiques. Le liquide intestinal de cheval, si étrange que le fait puisse paraître, ne varie par énormément sa concentration moléculaire.

Nous nous sommes donc demandé si pour des changements faibles de concentration, lors des conditions physiologiques, l'*Ascaris* répondait par des modifications de poids. Pour cela, nous avons utilisé le liquide intestinal de cheval. Il est filtré sur laine de verre lâche, afin de le débarrasser des grosses particules végétales. On obtient ainsi un liquide visqueux qui est le liquide intestinal normal. Il importe d'avoir un liquide intestinal très frais, car surtout à 37° les putréfactions sont rapides. Il va de soi que le liquide intestinal utilisé provenait d'un cheval parasité et que toutes les expériences ont été faites avec parasites et liquide intestinal correspondants; le liquide intestinal correspondait autant que possible à la situation des parasites dans l'intestin. Ce liquide est dilué à la moitié, au quart, au huitième, au seizième; nous avons ainsi cinq milieux dont les Δ sont respectivement:

N	*N*/2	*N*/4	*N*/8	*N*/16
− 0°,695	− 0°,42	− 0°,21	− 0°,12	− 0°,07



Graphique 2. Concentration du liquide intestinal en fonction de la dilution.

Expériences. Cinq *Ascaris* femelles vivants, extraits depuis peu de l'intestin de leur hôte, ont leurs extrémités caudale et buccale ligaturées avec un fil de soie, afin de limiter à la cuticule la régulation osmotique possible et pour éviter le vidage de l'intestin; ils sont plongés chacun dans l'un des cinq milieux préparés. Étuve à 37°, obscurité. Ils sont pesés à intervalles réguliers.

Résultats obtenus (augmentation ou diminution de poids):

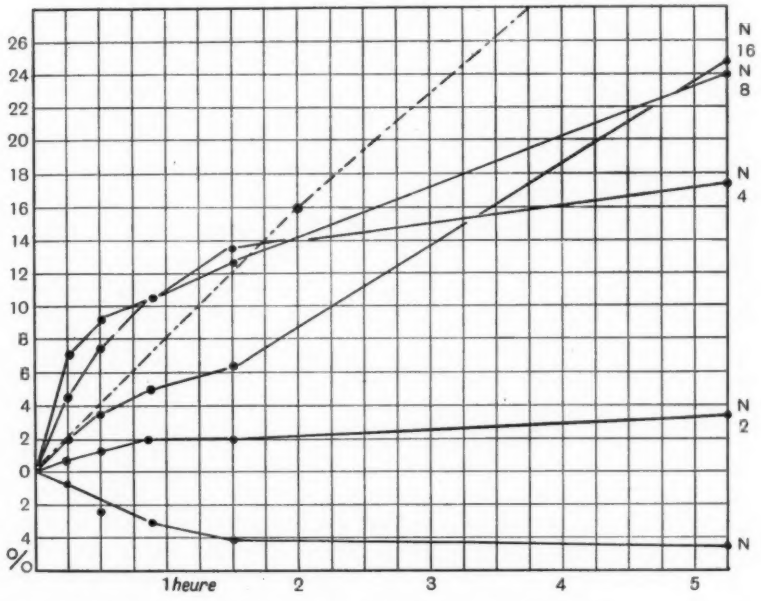
TABLEAU I.

Après	0 h. 0 m.	15 m.	30 m.	1 h.	1 h. 30 m.	5 h. 15 m.
N	12,55 gr.	12,45	12,25	12,20	12,05	12
N/2	12,45	12,55	12,65	12,7	12,7	12,9
N/4	10,35	10,85	11,15	11,45	11,75	12
N/8	8,35	8,90	9,15	9,25	9,40	10,4
N/16	11,65	11,95	12	12,15	12,40	14,50

TABLEAU II.

(*Augmentation et diminution de poids en* %.)

Après	0 h. 0 m.	15 m.	30 m.	1 h.	1 h. 30 m.	5 h. 15 m.
N	—	0,79	2,27	2,76	3,95	4,34
N/2	—	0,8	1,6	2	2	3,60
N/4	—	4,75	7,76	10,67	13,5	17,9
N/8	—	7,14	9,52	10,71	12,5	24,39
N/16	—	2,50	2,57	5,25	6,44	24,45

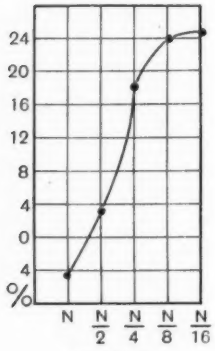


Graphique 3. Augmentations en %, en fonction du temps. Dans tous les milieux les animaux sont vivants après cinq heures. Seul l'animal de *N*/16 est en mauvais état.

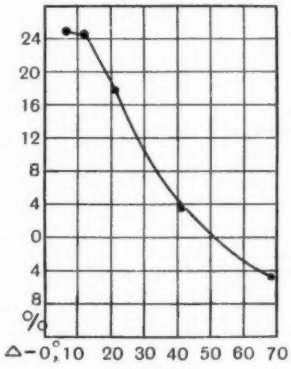
Les expériences sont arrêtées avant que le maximum de poids et le maximum de tension élastique de la cuticule soient atteints. Les courbes que nous donnons aujourd'hui ne représentent que la première partie de la courbe d'endosmose, le début du phénomène.

L'augmentation de poids dans les solutions de liquide intestinal dilué est donc certaine. Les augmentations de poids semblent proportionnelles à l'hypotonie:

N	*N*/2	*N*/4	*N*/8	*N*/16
− 4,34 %	+ 3,60	+ 17,9	+ 24,39	+24,45



Graphique 4.



Graphique 5.

Graphique 4. Augmentation de poids, après cinq heures en fonction de la concentration du milieu (exprimée en *N*).
Graphique 5. La concentration du milieu est exprimée par Δ[1].

Seule la courbe de *N*/16 semble débuter d'une façon anormale, mais il est probable que si nous avions poursuivi les pesées jusqu'à l'établissement du maximum et l'atteinte de la période de stabilité, cette anomalie aurait disparu. Au-dessus des courbes obtenues dans cette expérience, nous avons placé en pointillé une courbe obtenue précédemment avec l'eau distillée (hypotonie maximum); elle présente l'augmentation de poids maximum; mais le choix de cette courbe est arbitraire car nous avions d'autres courbes d'hypotonie maximum dont le maximum de poids était inférieur à ceux que nous obtenons aujourd'hui. Il est probable que si nous avions continué les pesées assez longtemps la proportionnalité se serait vérifiée également. Cependant, nous avons déjà fait remarquer qu'il ne faut pas s'attendre à obtenir des courbes que l'on puisse sérier exactement, selon l'hypotonie du milieu; car d'autres facteurs interviennent. Deux animaux de même poids placés dans une même solution peuvent avoir une courbe d'endosmose différente, si la tension élastique de leur membrane et la quantité absolue de liquide qu'ils contiennent sont différentes. De toute façon, pour diminuer les causes d'erreurs, il importe que les différents *Ascaris* proviennent du même cheval. Les conditions de notre expérience ne sont pas encore exactement les conditions physiologiques puisque nous avons ligaturé les deux extrémités et éliminé le rôle possible de

[1] Ces graphiques ne sont naturellement valables que pour la cinquième heure.

l'intestin. Nous pouvons déjà supposer que les phénomènes ne seront pas changés si l'intestin intervient dans la régulation osmotique. Tout au plus seront-ils accélérés puisque les surfaces d'absorption sont plus considérables. Si la solution extérieure pénètre dans le canal intestinal, il y aura endosmose au niveau de ce dernier et gonflement; la lumière de l'intestin sera de plus en plus réduite, et le contenu de l'intestin expulsé. En examinant les courbes obtenues dans notre travail précédent, en opérant avec des *Ascaris* non ligaturés, nous voyons que l'allure du phénomène n'est pas notablement modifiée.

Dans le liquide intestinal normal, il y a une faible diminution de poids indiquant que ce liquide est légèrement hypertonique par rapport au liquide cœlomique. Cela tient peut-être au fait que le liquide intestinal recueilli ne correspondait pas exactement à la situation des *Ascaris* dans l'intestin ou qu'il s'est légèrement concentré depuis le prélèvement. Si nous prenons la moyenne entre N et $N/2$ nous trouvons un Δ de $-0°,56$. Dans ce liquide il devrait y avoir normalement stabilité de poids. Il serait approximativement isotonique avec le liquide d'*Ascaris*. Cependant, comme l'équilibre qui s'établit entre le liquide cœlomique et le milieu extérieur doit être complexe, il n'est pas certain que la stabilité de poids se manifeste dans un milieu exactement isotonique avec ce liquide cœlomique[1].

De toute façon il est maintenant certain que la régulation osmotique se produit dans les conditions physiologiques. Nous confirmons nos précédents résultats: l'augmentation de poids est bien due à une pénétration d'eau qui dilue le liquide cœlomique; nous en avons la preuve par la forte turgescence de l'animal et la forte tension de la cuticule. Pour être complet, nous aurions dû mesurer les concentrations moléculaires des liquides d'*Ascaris* ayant séjourné dans les différentes solutions. Cependant, nous n'avons pu le faire, la quantité de liquide fournie par chaque animal étant trop petite pour être cryoscopée; nous nous réservons de revenir sur ce point en utilisant un microcryoscope.

Teneur du liquide cœlomique en chlorure.

Marcet avait déjà remarqué la pauvreté de ce liquide en chlorure; il ne donne pas de chiffres. Nous avons titré les chlorures par une méthode très précise (méthode de Volhard, modifiée par Laudat).

2 cc. de liquide cœlomique.
5 cc. $AgNO_3$ $N/10$.
4 cc. $KMnO_4$
5 cc. HNO_3 conc.

Le mélange est chauffé jusqu'à décoloration complète, la matière organique est détruite; $AgNO_3$ en excès est titré par KCNS en présence d'alun de fer ammoniacal. Nous trouvons comme moyenne de 5 analyses une teneur de

[1] Dans notre précédent travail, nous avions émis l'hypothèse qu'entre le liquide intestinal de l'hôte et le liquide cœlomique d'*Ascaris* s'établissait un équilibre de Donnan. Nous sommes obligés d'abandonner cette hypothèse, car, pour l'instant, la démonstration de ce fait est impossible.

1,20 $^0/_{00}$ ce qui est très faible. Dans un cas, 2½ c.c. de liquide cœlomique provenant de 3 *Ascaris* du même cheval avait une teneur en chlorure de 1,17 $^0/_{00}$. Le liquide intestinal correspondant, une teneur de 4,68 $^0/_{00}$.

Si nous calculons l'abaissement du point de congélation dû à 1,20 $^0/_{00}$ de chlorure exprimé en NaCl, nous trouvons:

$$\Delta = m \times 1{,}85 \times i$$

$M = 0{,}0205$ mol. gr.; $i = 2$.

$$\Delta = 0{,}0205 \times 1{,}85 \times 2$$
$$\Delta = -0°{,}076.$$

Les chlorures ne contribuent donc que faiblement à l'établissement de la pression osmotique totale (due aux électrolytes seulement).

Si nous prenons comme moyenne du liquide cœlomique $\Delta = -0°{,}62$, nous trouvons que le rapport de la pression osmotique due au chlorure, à la pression osmotique totale n'est que de 0,12.

Note. Les Δ sont exprimés en degrés centigrades. Nous avons indifféremment employé les termes concentration moléculaire et pression osmotique; quoiqu'ils désignent deux choses différentes, ils sont synonymes si l'on adopte la définition classique du terme pression osmotique et si l'on ne considère que la pression osmotique due aux électrolytes seuls.

Conclusions.

Les résultats obtenus précédemment en opérant dans des conditions artificielles se vérifient dans les conditions physiologiques; aux variations de concentration du liquide intestinal l'*Ascaris* répond par une augmentation de poids (dilution du liquide cœlomique) correspondante. Il semble donc soumis à toutes les variations de concentration de son milieu.

La teneur en chlorure est de 1,20 $^0/_{00}$. Ces chlorures ne participent que pour 0,12 à l'établissement de la pression osmotique totale.

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ON THE CULTIVATION OF *ENTAMOEBA HISTOLYTICA* AND SOME OTHER ENTOZOIC AMOEBAE.

By CLIFFORD DOBELL and P. P. LAIDLAW.

National Institute for Medical Research, London, N.W. 3.

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I. Introduction.

During the last half-century many attempts have been made to cultivate *Entamoeba histolytica* and other entozoic amoebae in artificial media: but though numerous claims to success have from time to time been made, it is only very recently that any methods have been devised which are at once sufficiently authenticated to be credible and sufficiently straightforward to admit of ready confirmation by workers other than the original claimants. Most, if not all, of the earlier "successes" are now known to have rested upon a misidentification of the organisms cultivated, or to have involved other familiar fallacies of observation or interpretation: and as none of them has yet been substantiated, or been able to bear critical later examination, it now seems unnecessary to review the history of this subject anew.

Apart from certain debatable results announced by Cutler (1918)[1], the first manifestly successful attempt to cultivate any entozoic amoeba *in vitro* appears to be that of Barret and Smith (1924), who cultivated two intestinal species of *Entamoeba* from cold-blooded vertebrates—*E. barreti* from turtles, and *E. ranarum* from frogs. Their cultures of the first species were independently examined by Taliaferro[2] and Holmes (1924), with entirely confirmatory results; while a fuller account of the cultivation of *E. ranarum* has just been published by Barret and Smith (1926) and verified by Taliaferro and Fisher (1926).

[1] Cf. p. 295 *infra*.

[2] Dr W. H. Taliaferro, at the time, kindly sent preparations of *E. barreti* (from the turtle and from cultures) to one of us (C. D.), and these substantiated the statements of Barret and Smith convincingly.

While the method devised by Barret and Smith appears to be adequate for the species which they studied, it does not seem to be adaptable to the cultivation of all entozoic amoebae[1]. But almost immediately after[2] the appearance of their first publication, Boeck and Drbohlav (1924) announced that they had succeeded in cultivating *Entamoeba histolytica* by an entirely different method; and this claim, as is now well known, they were subsequently able to establish (see Boeck and Drbohlav (1925, 1925 *a*), Drbohlav (1925, 1925 *a*, 1925 *b*), etc.). Since the publication of their early papers many independent workers have been able to confirm their results. We ourselves have cultivated *E. histolytica* and various other entozoic amoebae by similar methods daily since November, 1924.

It is now clear that the Boeck-Drbohlav technique constitutes a great advance in experimental protozoology. The method is not merely a special one for the cultivation of a single intestinal species (*E. histolytica*), but a general method—when appropriately modified—for the artificial maintenance *in vitro* of many different entozoic protozoa; and it thus affords a most valuable aid to the study of a great variety of problems. But certain difficulties and imperfections in the original procedure are soon evident to anyone who tries it, and these we have attempted—for a particular purpose—to obviate. Some of our results are described in the present paper. In certain directions we have, we think, made definite advance: in others we have met with failure. But even when our experiments have been wholly negative they sometimes seem worthy of record, since their publication may prevent others from pursuing unprofitable lines of investigation. Our work is still in progress; and our results, therefore, are not final.

We desire, at the outset, to offer our sincerest thanks to Dr William C. Boeck (of Harvard) and Dr Jaroslav Drbohlav (of Prague), the originators of the methods which we have endeavoured to exploit. Dr Boeck, at the end of 1924, generously sent one of us (C. D.) a copy of their joint paper (1925)—then unpublished—together with numerous confirmatory preparations and photomicrographs. Dr Drbohlav, in November, 1924, during his stay in London, not only demonstrated their methods to us personally, but also gave us cultures of a strain of *E. histolytica* which we have maintained and experimented with ever since. The advice and information which he also then gave us have proved invaluable. To both these workers, therefore, we here wish to acknowledge, to the fullest extent, our deep indebtedness.

II. The Amoebae cultivated during the Present Investigations.

Boeck and Drbohlav (1925) were originally able to cultivate only one species of intestinal amoeba—*Entamoeba histolytica*. But it appeared probable, from their findings, that *Entamoeba coli* and other entozoic amoebae could

[1] Some experiments in this connexion will be described later. See p. 296 *infra*.

[2] Barret and Smith's paper was published in May, 1924, while the first announcement by Boeck and Drbohlav was made in June of the same year.

be cultivated in a similar manner, if studied with equal care: and this was soon borne out by the later work of Drbohlav, who successfully adapted the original technique to the cultivation of *E. coli* (Drbohlav, 1925 *e*), *E. gingivalis* (1925 *d*), "*E. dysenteriae* type *tetragena minuta*[1]" (1925 *c*), and *E. aulastomi* (1925 *f*).

Meantime many other workers had been able to cultivate *E. histolytica* by adopting the same methods, and some of them have already recorded their results (Chiang (1925), Andrews (1925), Gupta (1925), Guérin and Pons (1925, 1925 *a*), Thomson and Robertson (1925), etc.). Chiang (1925) further reports the successful cultivation of "*E. histolytica murina*" (from rats): Gupta (1925) has obtained cultures of "*E. nuttalli*" (from *Macacus rhesus*): while Thomson and Robertson (1925) announce successes with *E. coli*, *Dientamoeba fragilis*, and *Endolimax nana*, and report "the probable cultivation" of *Iodamoeba bütschlii* (all from man).

Our own work, which was begun in November 1924, has been chiefly concerned with the cultivation of certain entozoic amoebae from man and monkeys. It was undertaken partly with the object of carrying out various chemotherapeutic tests—which are reported elsewhere (Dobell and Laidlaw, 1926)—but more particularly as a preliminary to a series of researches in which one of us (C. D.) is at present engaged. This accounts for our choice of material, which was not selected for any other reasons. We have now cultivated many strains of amoebae, belonging to several different species; so before describing our methods it will be convenient to note more particularly what these species are, and how they have been obtained. The following is a brief list of them[2], with various relevant details.

(1) *Entamoeba histolytica* from man. Two strains have been specially studied—one (Strain D) originally cultivated and given to us by Dr Drbohlav[3], the other (Strain F) isolated by one of us (C. D.) later.

Strain D was isolated from an acute case of human amoebic dysentery on 3 November, 1924. It was handed to us at the 11th subculture on 24 November, 1924, and we have maintained it ever since (up to the time of writing, for 18 months—172 serial subcultures). It is still growing luxuriantly, and has been used for many experiments.

Strain F was isolated on 25 August, 1925, from the faeces of a patient[4] who was not at the time suffering, and had never in the past suffered, from dysentery. He displayed symptoms of intestinal sepsis which rapidly abated

[1] This is the name given by Drbohlav (1925 *c*) to the species which he studied. We presume that he means by it the organism variously regarded as a strain of *E. histolytica* forming small cysts, or as a distinct species of human intestinal amoeba ("*E. hartmanni*" or "*E. tenuis*"). Cf. Dobell (1919).

[2] For the nomenclature of these species see Dobell (1919), where the synonymy and more detailed descriptions will also be found.

[3] See Drbohlav (1925, 1925 *b*), where its origin is described.

[4] We are indebted to Dr H. C. Sinderson, who was in charge of this patient, for this material and also for the clinical information.

after vigorous treatment with emetine, which apparently cured his amoebic infection[1]. The original strain (F) was cultivated until 18 September, 1925 (14 serial subcultures), when it was voluntarily abandoned: but one of the early cultures was inoculated into a kitten, which developed acute amoebic dysentery 3 days later; and from this animal, before it was killed, several substrains were isolated. One of these was maintained from 1 September, 1925, until 10 February, 1926 (34 serial subcultures), when it was voluntarily given up—though growing excellently. We performed comparatively few experiments with this amoeba—most of them having been undertaken with the object of checking those previously obtained with Strain D.

Both these strains (D and F) of *E. histolytica* appeared to be typical in every way. Both displayed the characteristic morphology of the species; both—under suitable conditions—formed typical quadrinucleate cysts; both were able to cause acute amoebic dysentery in kittens (at an early stage in their history *in vitro*)[2]; and both ingested human red blood-corpuscles readily, when tested in cultures. Furthermore, we have ascertained that both these strains were highly sensitive to emetine *in vitro* (Dobell and Laidlaw, 1926), and one at least (F) was apparently eradicated by this alkaloid from its original human host.

(2) "*Entamoeba histolytica*" from the faeces of monkeys of the genus *Macacus*. Although we have now cultivated and studied a large number of strains of this species (from *M. rhesus* and *M. sinicus*), we are still in doubt regarding its correct specific designation. Morphologically and culturally all the strains studied appear to belong to a single species, which is not distinguishable with certainty from *Entamoeba histolytica* (of man) by any valid systematic character. During the last two years one of us (C. D.) has made a detailed study of this amoeba; but as this work is still in progress, we are not yet in a position to draw any final conclusions. We will only add that this species is probably identical with that previously named *E. nuttalli* by Castellani and *E. duboscqi* by Mathis, and that it has been variously designated by other workers (cf. Dobell (1919), p. 131). Some strains are capable of infecting kittens and causing amoebic dysentery in these animals[3]; and all—so far as we have tested them—are highly sensitive to the action of emetine in cultures (Dobell and Laidlaw, 1926).

We have isolated and cultivated this species on numerous occasions from 3 individuals of *Macacus rhesus* and 4 of *M. sinicus*, and we have also recovered it in culture repeatedly from experimentally infected kittens. We are now able, indeed, to cultivate it at will—in pure culture—from any infected animal, and to maintain it *in vitro* for as long as we desire. We have, however, studied more particularly two strains—one (J) isolated on 24 November, 1924,

[1] One of us (C. D.) examined the stools of this patient, both microscopically and culturally, on the 15th, 16th, 17th, and 18th days after the end of treatment. The results were consistently negative throughout.

[2] See p. 314 *infra* for further details.

[3] Unpublished observations (C. D.).

from *M. rhesus* and voluntarily abandoned on 18 May, 1925 (after 50 serial subcultures), the other (S) from *M. sinicus*, isolated on 29 December, 1924, and voluntarily relinquished on 25 May, 1925 (51 serial subcultures). Numerous experiments have been made with both these strains. At the time when they were given up they were both growing extremely well, and could have been propagated further for any desired period.

(3) *Entamoeba gingivalis*, from the human mouth. We have cultivated only one strain of this species. It was isolated from a fragment of tartar—scraped off a sound tooth in the mouth of a healthy man, who was not suffering from pyorrhoea or other dental disease—on 26 June, 1925; and after 22 serial subcultures it was voluntarily given up on 11 September, 1925. At this time the strain was growing excellently in pure culture, and could have been maintained indefinitely.

We performed many experiments with cultures of this strain, but have made no attempt to isolate and study others.

(4) *Entamoeba coli*, from human faeces. We found unexpected difficulty in isolating pure cultures of this species; but after repeated failures we succeeded—by means of a special technique, which will be described later—in obtaining a strain which now grows with great freedom, and which we are able to propagate without difficulty. This strain (λ) was started from typical 8-nucleate cysts in the stool of a man—not suffering from any intestinal disease—on 24 November, 1925, and has been maintained in culture ever since. At the time of writing (May, 1926) it is at the 36th serial subculture. Many experiments have already been made with it, and others are in progress. It is morphologically a typical *E. coli*, forming 8-nucleate cysts and displaying all other characteristics of the species.

(5) "*Entamoeba coli*" from the faeces of a monkey (*Macacus sinicus*). This species is morphologically identical with *E. coli* of man, and appears to be the same as that described previously, from other monkeys, as "*E. pitheci*," "*E. legeri*," etc., by various workers[1]. We have cultivated it, along with other species, on several occasions; but in spite of numerous attempts we have not yet been able to isolate it in pure culture, and will therefore merely note here that it appears to be culturally identical with *E. coli* from man—so far as we have ascertained. Further work on this organism is in progress.

(6) *Entamoeba* sp., from *Macacus rhesus* and *M. sinicus*. This is an extremely small species—present in every monkey examined—which closely resembles the organism found in man and variously regarded as a small race of *E. histolytica* (with 4-nucleate cysts, 7 μ–9 μ in diameter) or as a distinct species called "*E. hartmanni*," "*E. tenuis*," etc.[2]. At present we cannot name the form from monkeys more exactly. (It is possibly *E. chattoni* Swellengrebel, 1914.)

[1] Cf. Dobell (1919), p. 131.

[2] Cf. Dobell (1919). The form from monkeys is hardly—if at all—distinguishable from the amoeba of man already cultivated by Drbohlav (1925 *c*) and designated by him "*E. dysenteriae* type *tetragena minuta*."

We have been able to cultivate this species for various periods on numerous occasions, in company with others: but as we have only recently succeeded in isolating it in pure culture—after repeated failures—we have not yet studied it satisfactorily. At the time of writing we have cultivated it for 55 days (32 serial subcultures). Its cultural requirements are closely similar to those of *E. nana* and *E. histolytica*. Experiments with it are being continued.

(7) *Endolimax nana*, from the faeces of man and monkeys (*M. rhesus* and *M. sinicus*). One of us (C. D.) has already performed a long series of experiments with this organism—the results of which will be described elsewhere. We will only note here that many pure cultures have been obtained and studied in detail, and that the chief cultural requirements of the species have been ascertained. Strains isolated from monkeys and from man all appear to be identical in every way, and we now have no hesitation—as a result of our experiments[1]—in assigning them all to the same species. Of the various strains studied, the most important is one (Σ) isolated from *M. sinicus*. This was maintained from 21 July, 1925, until 31 January, 1926 (51 serial subcultures), when it was voluntarily abandoned—growing luxuriantly, and cultivable *ad libitum*. Many other strains (from man and monkeys) have been kept for shorter periods.

All strains of *E. nana*—when obtained pure—have been morphologically typical in every way, and under suitable cultural conditions have formed characteristic 4-nucleate cysts.

III. The Composition and Preparation of Culture-Media.

The medium in which Boeck and Drbohlav (1924) first succeeded in cultivating *E. histolytica* was one which Boeck had earlier devised for the cultivation of intestinal flagellates. Numerous modifications were afterwards prepared and tested by these workers—together or separately. (See especially Boeck and Drbohlav (1925), and Drbohlav (1925 *b*).)

All these media are made on the same principle. They consist of two parts—solid and liquid. The solid is prepared in the form of ordinary bacteriological slopes: the liquid is then added so as to cover it more or less completely. In the original formula the solid constituent was made from whole eggs thoroughly emulsified with Locke's solution (4 eggs to 50 c.c. of liquid), and then coagulated by heat (70° C.). To this, after sterilization in the autoclave, the liquid constituent—previously sterilized by filtration—was added. It consisted of inactivated serum (human or horse) diluted with Locke's solution (1:8). Boeck and Drbohlav (1925) call this mixture "L.E.S. medium" (*i.e.* Locke-Egg-Serum); and they found that its initial reaction "varied from *p*H 7·2 to 7·8 and needed no adjustment"—this being the optimum range of hydrogen-ion concentration determined by them for *E. histolytica*.

[1] To be published shortly (C. D.).

Material containing the amoebae to be cultivated is introduced into the previously warmed medium by means of a sterile capillary pipette. It sinks to the bottom—at the junction of the solid and liquid with the glass—and there the amoebae chiefly develop when the tubes are incubated at 37° C. under aerobic conditions. Maximum growth is usually seen on the second day of incubation. On the fourth or fifth day the amoebae usually die out, unless they are transplanted into a similar subculture. Under such conditions they do not encyst; but with frequent transplantation they can be propagated in the free amoeboid form for an unlimited period.

It is obvious that a medium of this type can be modified in many ways. The solid and liquid constituents can be varied, and various substances can be introduced into the cultures along with the amoebae. Many such modifications were tried by the inventors of the method: and they finally recommended the employment of dilute egg-white (1 per cent. crystallized egg-albumin in Ringer's or Locke's solution, or the white of one egg to the litre of liquid) in place of serum, and of ordinary blood-agar in place of coagulated egg. All possible combinations of these various solids and liquids appear to suffice for the cultivation of *E. histolytica*.

In a recent communication Drbohlav (1925 *b*) particularly advocates N.N.N. blood-agar covered with Ringer-eggwhite which has been buffered by the addition of 5 gms. per litre of KH_2PO_4 and adjusted to a *p*H of 7·4; and a similar medium in which the solid consists of agar (1·4 per cent.) in which 1 per cent. of dissolved starch has been incorporated.

Our own experiments have been made with three special objects in view. We endeavoured to modify the original media (1) so that richer cultures could be obtained; (2) so that the amoebae would live and multiply in individual cultures for a longer period than two or three days; and (3) so that they would complete their life-history in the test-tube—finally forming cysts. The first two objects we have, in a satisfactory measure, achieved: in the last we have often succeeded but more often failed. Our experiments in this connexion are described in the next section.

Another series of experiments was undertaken to ascertain the conditions necessary for initiating cultures from cysts—not free amoebae. This is an important practical aspect of the subject which has not hitherto been adequately studied, but we shall consider it separately in a later section (see p. 302 *infra*).

We have tried many variants of the original Boeck-Drbohlav medium—experimenting with all the amoebae listed in the preceding section. As the solid constituent we have especially used emulsified and coagulated whole egg, inspissated whole blood or serum, and agar (with various modifications). The liquids tried have been chiefly egg-albumin or serum, diluted with various fluids in varying proportions and containing a variety of dissolved salts and other substances; ascitic fluid; gum arabic solutions; and watery extracts and infusions of hay. Most of the possible combinations of these solids and

liquids have been used as culture-media, and have been found more or less satisfactory for some at least of the strains of amoebae studied. For general purposes, however, we now rely—as a result of our experience—almost entirely upon two solids (coagulated whole egg or inspissated horse-serum), and two liquids (dilute egg-albumin or dilute horse-serum). But we prepare and supplement these mixtures in a manner which profoundly affects their utility.

It will be recalled that the original medium of Boeck and Drbohlav consisted of coagulated egg (emulsified in Locke's solution) covered by dilute serum or egg-albumin. Now coagulated egg and simple egg-albumin—especially when both constituents are diluted with Ringer's fluid—form a medium obviously deficient in carbohydrate. When Locke's solution is used instead of Ringer's—as in the original formula[1]—this deficiency is partly made good. The contained dextrose, however, is readily split—with the production of acid, which is highly detrimental to the amoebae—by many of the intestinal bacteria which are unavoidably present in amoebic cultures. Consequently, it seems doubtful how far even the small amount of sugar present in such media can be directly utilized by the amoebae. But many intestinal amoebae normally ingest solid starch and most of them store glycogen in their cysts. They thus seem to require more carbohydrate than the media afford; and it seemed reasonable to suppose, therefore, that *E. histolytica* would have a better chance of growing and encysting *in vitro* if a more adequate supply of carbohydrate were available.

At the beginning of our work it occurred to us that *solid starch*, if added to the *liquid*, might satisfy the requirements of *E. histolytica*. If the amoebae would ingest it, they would obtain abundant carbohydrate; and they would obtain it in a form in which it would be accessible to them alone, without the risk of a simultaneous production of acid by bacteria in the medium. When we first tried this experiment[2], the results exceeded our most sanguine expectations. We found that *E. histolytica* readily ingested solid starch; that the amoebae then multiplied enormously; and that they finally stored up abundant glycogen in their protoplasm and encysted—thus completing their life-cycle. No concomitant production of acid occurred, and the amoebae lived much longer than they did in Boeck-Drbohlav medium.

The effect of adding solid starch to a culture of *E. histolytica*, *E. coli*, or *E. gingivalis*, is often, indeed, astonishing. Within 24 hours a previously poor culture is teeming with amoebae—most of them gorged with starch-grains in various stages of digestion. We soon found, however, that the preparation of a suitable starch-containing medium is not such a simple matter as at first sight it might appear, and we met with many obstacles in our attempts to perfect our original technique. For example, we found that it is important

[1] The "Locke's solution" of Boeck and Drbohlav contains 0·25 per cent. of dextrose.

[2] The experiment was first made by one of us (P. P. L.) with *E. histolytica* (Strain D) on 1 Dec. 1924.

to obtain an appropriate kind of starch: the grains must be sufficiently small for the amoebae to be able to ingest them. Again, it is not easy to sterilize solid starch without making it soluble. And finally—and this is a point of great practical importance—in many stools and cultures containing entozoic amoebae, other micro-organisms are also present which are capable of rapidly fermenting undissolved starch, with the production of acid; and consequently, if starch be added to a culture containing them the result is not a rich growth of amoebae but a strongly acid solution in which no amoeba can live. We therefore had to ascertain (*a*) which kinds of starch are most suitable: (*b*) how these starches can be sterilized: and (*c*) how the splitting of this starch, by concomitant bacteria, can be prevented. We made many experiments in order to find answers to these questions.

(*a*) *The best kind of Starch.*—We have tried twelve different natural starches (pure samples, controlled by microscopical examination): namely, that from Rice, Wheat, Buckwheat, Sago, Peas, Barley, Tapioca, Maize, Oats, Potatoes, Rye, and Arrowroot. We found that Potatoes, Sago, Peas, Barley, Tapioca, and Arrowroot contain grains too large for the amoebae to engulf them. Starch from these sources, therefore, is practically worthless. Oats, Rye, Maize, and Buckwheat, however, contain many appropriately minute grains. But by far the most suitable starch is that from *Rice*. This can be obtained in great purity, and is almost entirely composed of uniformly small grains which the amoebae can easily take up and assimilate. We therefore now use this exclusively.

(*b*) *Sterilization of the Starch.*—After various trials we have found—contrary to the indications in some works which we have consulted—that solid rice-starch can be completely sterilized without being charred, disintegrated, or rendered soluble, by subjecting it to a temperature of 180° C. (dry heat) for one hour. It should be dry, before heating, and heated in small quantities—not in bulk. (We sterilize it loosely packed in small tubes containing about 2·5 gms. each.) All the rice-starch used in our media is now treated in this manner.

(*c*) *Eradication of Starch-splitting Bacteria.*—In order to remove organisms which are able to ferment solid starch, we tried to discover some substance which, when added to the medium, would kill them off without injuring the amoebae. Although we have experimented with many dyes and other chemicals, we have so far found only one substance which ever answers this purpose. This is the yellow dye Flavine (otherwise known as trypaflavine, or acriflavine), which we have found to be harmless, in dilute solution, to *E. histolytica* and most of the other amoebae studied, but very poisonous to the majority of starch-splitting bacteria which we have encountered. *E. histolytica* will withstand flavine in a concentration as high as 1 part in 10,000; while the addition of 1 part in 20,000 (maintained, if necessary, for several subcultures) will usually kill all starch-splitting bacteria. *E. coli* and *E. gingivalis* will also tolerate similar concentrations; but *Endolimax nana* appears to be more sensitive to the dye, and has generally been killed by 1 part in 20,000.

We have not attempted to isolate and identify the starch-splitting bacteria[1] which we have met with from time to time in our cultures. These organisms—whatever they may be—are not uncommon in the faeces of men, monkeys, and kittens: and when attempts are made to isolate amoebae from any of these sources in starch-containing media, they sometimes give considerable trouble. Prompt transplantation (for one or two subcultures) into medium containing flavine (1: 20,000) will usually remove them: but sometimes this treatment is unavailing, as the starch-splitters survive a higher concentration than the amoebae; and for such cases—which are not common, in our experience—we know of no remedy.

A good—or rather bad—example of such a persistent starch-splitting organism is furnished by our numerous failures to isolate *E. coli* from a particular individual. This man is infected with this amoeba only, and therefore appeared to be a suitable source from which to obtain a pure culture. But in spite of repeated efforts, for more than a year, to cultivate the amoebae directly from his stools, we never succeeded in doing so. Cultures made into ordinary media (without starch) were almost immediately extinguished by the rapid development of *Blastocystis* and acid-producing organisms (unidentified): while cultures containing starch were impossible to maintain for more than one or two generations. Invariably the starch was rapidly split, with the formation of much acid, and the amoebae soon died; while the starch-splitting organisms, in this case, were always able to withstand a higher concentration of flavine than the amoebae. At present, therefore, we do not know how it is possible to cultivate *E. coli*—in the amoeboid form—from this particular case. We have had one similar experience in trying to cultivate "*E. histolytica*" (simian strain) from a kitten, and several like failures in attempts to cultivate amoebae from monkeys.

Addition of flavine to cultures of entozoic amoebae often modifies the accompanying bacterial flora profoundly, and not always in the desired direction. Usually, in cultures containing this dye, the liquid is exceptionally limpid (owing to suppression of the bacteria), and the amoebae grow but slowly. Many bacteria are killed and stained yellow; but the amoebae ingest these readily, without evident injury. One of our lines of *E. histolytica* (Strain F) became almost uncultivable, however, after growth in flavine. The dye killed nearly all the original organisms, leaving the amoebae and a slime-forming bacillus: and thereafter each culture consisted of a lump of slime—at the bottom of the tube—in which masses of amoebae were involved. The slime was so tenacious that it could easily be drawn out into threads more than a yard long; and transplantation of these cultures became so difficult, in consequence, that this line was soon abandoned.

The media which we now use, and strongly recommend, for the cultivation

[1] One of them is a fusiform bacterium, which was present originally in our Strain D of *E. histolytica* (from man). It gave us great trouble soon after we began to grow this strain in starch-containing media, but was ultimately eliminated by the action of flavine.

of *E. histolytica* (all strains—human and simian), *E. coli*, and *E. gingivalis*, are therefore similar to those of Boeck and Drbohlav but modified by the addition of solid rice-starch to the liquid. Our egg-medium (solid) is made up with Ringer's solution[1]—not Locke's, which contains dextrose—and the covering liquid is egg-albumin or serum diluted with the same fluid. But before introducing the amoebae we add (by means of a platinum loop or spatula) a small amount of sterile solid rice-starch. This soon falls in a little white heap to the bottom of the tube: and in this heap, or on its surface, the amoebae inoculated afterwards develop.

We have found, moreover, that the egg (solid) may, with great advantage to many strains of amoebae, be replaced by inspissated horse-serum; and we now use this medium extensively, and prefer it to all others for general purposes. The solid serum may be covered with either dilute egg-white or dilute serum—as with the egg-media.

The preparation of egg-media has already been adequately described by Boeck and Drbohlav, but it will be necessary to describe the preparation of media having solid serum as a basis in more detail. Ours are made as follows:

Whole horse-serum (sterilized by filtration) is tubed in suitable amounts, with all aseptic precautions, and set in the form of ordinary slants by heating in an inspissator at 80° C. The time required to solidify the serum sufficiently is from one hour to one hour and ten minutes. *It is most important not to heat the serum longer*, for over-heating ruins it as a culture-medium. When the slants have cooled, they are incubated to test their sterility, and then covered with dilute serum or egg-albumin (sterilized by filtration). The mixed medium is then again incubated to make sure that it is sterile, and sterilized starch is added immediately before use.

We have found it advantageous to use a stronger solution of egg-albumin than that recommended by Drbohlav. We now make ours by carefully mixing the whites of four eggs in a litre of sterile Ringer's fluid, and then finally sterilize it by filtration through a Seitz filter. One great advantage of this stronger solution is that it enables wet films of the amoebae to be fixed more easily for cytological study.

It is unnecessary to adjust the initial reaction of medium made in this way. It is approximately neutral, and the inspissated serum itself appears to act as an effective buffer. If the *p*H of a culture of amoebae in such medium is experimentally altered by the addition of small amounts of acid or alkali, it rapidly readjusts itself to its original reaction on incubation (usually within a few minutes). This property probably accounts, to a large extent, for the particular virtue of inspissated (not over-heated) horse-serum as a basis for cultures of entozoic protozoa.

[1] The "Ringer's solution" which we use is prepared by dissolving 9 gms. NaCl, 0·2 gm. KCl and 0·2 gm. $CaCl_2$ in 1 litre of distilled water. It differs, therefore, from that employed by Drbohlav (1925 *b*).

Cultures of amoebae in medium composed of inspissated horse-serum + Ringer-eggwhite + rice-starch are very much richer and last much longer than those of the same organisms in the original Boeck-Drbohlav medium. *E. histolytica* (and the closely allied form from *Macacus* monkeys) usually lives for at least a week and often for 10 days—sometimes for a fortnight. Subcultures need not be made, therefore, oftener than once a week. *E. coli* lives and grows equally well, and lasts for a similar period: but *E. gingivalis*—the only strain studied—had to be transplanted every 5 or 6 days, as the cultures rarely endured much longer. This medium is not so suitable for *Endolimax nana*, however, which does not need starch and grows better in the original Boeck-Drbohlav medium.

Two other practical advantages of starch-media are (1) that they are of great value for eradicating *Blastocystis*, and (2) that in them the amoebae often encyst. But these points will be considered in more detail later.

It will be evident that the two chief solids used (coagulated whole eggs and inspissated serum), and the two chief covering liquids (dilute serum and dilute egg-albumin), can be combined in four different ways. Moreover, each of these four types of medium can be augmented by the addition of solid starch to the liquid, and consequently eight different media are thus possible. We have used all these extensively in our work, but have found that they are not all equally suitable for all species of amoebae. Our experience with the four chief species studied is summarized in Table I, in which the following abbreviations are used—the solid constituent of the medium being denoted by capitals, the liquid by small letters:

HS = inspissated whole horse-serum.
E = coagulated whole egg, emulsified in Ringer's solution.
hs = horse-serum (inactivated) diluted with Ringer's solution (1 : 8).
re = egg-albumin diluted with Ringer's solution.
S_o = solid rice-starch—added to the liquid.

TABLE I.

Medium	Growth of *E. histolytica* (human or simian)	*E. coli* (human)	*E. gingivalis* (human)	*E. nana* (human or simian)
(1) HShs	Poor	Poor	Poor	Very good, but rapid
(2) HShs + S	Good, but rapid	Good, but rapid	Good, but rapid	Good
(3) HSre	Very poor	Little or none	None	None
(4) HSre + S	Abundant and prolonged	Abundant and prolonged	Abundant	Poor
(5) Ehs	Moderate	Moderate	Poor	Excellent
(6) Ehs + S	Abundant and prolonged	Abundant and prolonged	Moderate	Good
(7) Ere	Poor	Little or none	Poor	Excellent
(8) Ere + S	Abundant and prolonged	Abundant and prolonged	Moderate	Good

The medium designated Ehs is virtually the original Boeck-Drbohlav medium (made with Ringer's solution in place of Locke's). In the table "rapid growth" means that the cultures grow quickly and well, but soon die unless transplanted (usually after 2 or 3 days). "Prolonged growth" indicates that the amoebae live for many days (a week or more). In cultures in which the growth is said to be "none," the amoebae will live for a time but appear not to multiply, and soon die. Other terms will explain themselves.

It should be noted that the addition of *soluble starch* (starch paste, precipitated starch, etc.) to the liquid part of any of these media, has a decidedly harmful effect on the amoebae. The culture rapidly becomes acid—as a result of bacterial activity—and the protozoa soon die. For a similar reason Drbohlav's (1925 *b*) method of incorporating dissolved starch in the solid constituent (agar) appears far less satisfactory than our procedure.

Although most of our experiments have been made with media of the type just considered, we have also tried several others compounded on different principles. Two of these—the media of Cutler and of Barret and Smith—are worth special mention.

Cutler's Media.—Cutler (1918) claims to have cultivated *E. histolytica* in two different liquid media—one prepared from emulsified eggs (with or without the addition of blood), the other from peptone and an extract of boiled human blood-clot. We tried all these liquids again—prepared as described by him. The method adopted was to inoculate tubes of Cutler's media with amoebae from our own richly growing cultures, and then to attempt to propagate them by subcultivation in the same media. When—as frequently happened—the subsequent microscopical examination of these "cultures" was negative, we tested them further by reinoculation into our own media, which we knew by previous experience to be highly favourable for growth. In this way—coupled, of course, with microscopical examination—we were able to ascertain whether Cutler's media were equally suitable, and how long the amoebae could survive in them.

The results of several series of careful experiments may be summarized in a few words. We not only found it impossible to cultivate our strains of *E. histolytica* in any of Cutler's media, but we found that the amoebae could not even survive in them for more than a very short time—probably owing largely to the excessive growth of bacteria. After inoculation into any of these solutions, the amoebae did not multiply but soon died. Attempted subcultures in the same fluids never once succeeded: and after 24 hours' incubation of the initial cultures, it was only very rarely that reinoculation into favourable media gave a positive result. In other words, the amoebae almost invariably succumb in these media within a day, without multiplying or encysting. Yet *E. histolytica* will often survive for several days in pure distilled water, or Ringer's solution, under the same conditions—as we have determined by

experiment: and we conclude, therefore, that Cutler's media are not merely unsuitable for cultivating this species, but even harmful to it.

Our experiments thus entirely confirm the previous conclusions of Dobell and Douglas[1], and in no way support the more favourable interpretations (unsupported by experiments) of Barret and Smith (1924) and Boeck and Drbohlav (1925). Consequently, we think it legitimate to conclude that the latter workers were probably the first who ever really succeeded in cultivating *E. histolytica*.

The Medium of Barret and Smith.—Since Barret and Smith (1924, 1926) succeeded in cultivating two species of *Entamoeba* from cold-blooded vertebrates by a very simple method, we tried their medium for *E. histolytica* and several other amoebae. It consists of a 10 per cent. solution of serum in 0·5 per cent. sodium chloride. We have tried three kinds of serum (from horse, rabbit, and calf), and have also tried increasing the salt-content to 0·75 per cent.; but the results have been uniformly negative. When *E. histolytica*, *E. coli*, or *E. nana* is inoculated into such media, and the cultures are incubated at 37° C. (the temperature necessary for all these species), there is invariably a rapid growth of bacteria, and the amoebae soon perish. Sometimes they will live for several days; but the medium soon becomes acid, and we have found it impossible to obtain serial subcultures, even with frequent transplantation. The addition of solid starch somewhat improves the medium, but does not make it suitable for prolonged cultivation.

Moreover, we have found it impossible to isolate any species of amoeba[2] from faeces in the medium of Barret and Smith. As Barret (1921) has shown, it is very favourable for the cultivation of *Blastocystis*: and when stools are inoculated into it, the result, almost invariably, is a prodigious growth of this organism, with consequent extinction of the amoebae.

It seems clear, therefore, that media of this type are unsuitable for the isolation and cultivation of *E. histolytica* and other amoebae of warm-blooded vertebrates.

In conclusion it may be noted that we have found it possible to keep *E. histolytica* (from man and monkeys) alive for a long time in plain physiological saline or Ringer's solution to which some solid starch has been added. If the amoebae from a richly growing culture are transferred to such an environment, and kept at a temperature of 37° C., they will not multiply or encyst, but many of them will often survive for two or three weeks. For example, in one of our experiments with "*E. histolytica*" (Strain J, from *M. rhesus*) they were recovered (by cultivation) after a sojourn of 21 days in normal saline (0·75 per cent. NaCl) and starch; though a week later (28 days) they were all dead. Under such conditions *E. histolytica* will generally survive for about a fortnight, but the time varies with different strains.

[1] See Dobell (1919), p. 70.

[2] That is, of those which we have particularly studied (from man and monkeys). We do not question the results of Barret and Smith with amoebae from cold-blooded vertebrates.

Whilst cultivating our various strains of amoebae we repeatedly studied the effects of adding different substances to the media, and of varying the physical conditions. But as most of these experiments were undertaken with the special object of ascertaining the factors which determine cyst-formation in cultures, they can be most conveniently recorded in the next section.

IV. THE FACTORS DETERMINING ENCYSTATION IN CULTURES.

It will be recalled that Boeck and Drbohlav (1925) found that *E. histolytica*, when cultivated by their methods, did not encyst. Only once—with their "Strain E," 20 days after isolation—did they find cysts in their cultures, and even then only 6 were seen. Drbohlav (1925 *b*) has since made numerous attempts to induce encystation, but without success.

On the other hand, Chiang (1925) states that encystation occurred regularly in his cultures of "*E. histolytica murina*" when the original Boeck-Drbohlav medium was enriched with a small amount of broth and evaporation of the liquid occurred: while Guérin and Pons (1925, 1925 *a*) and Thomson and Robertson (1925)—all using the original method—state that, in their cultures, cysts were met with sporadically. Barret and Smith (1924) obtained no cysts in cultures of *E. barreti* (from turtles), but found (1926) that they were sometimes formed by *E. ranarum* under similar conditions.

We have already mentioned that when we first began to use media containing solid starch, many of the amoebae in our cultures encysted. They assumed the precystic form, made and stored much glycogen in their cytoplasm, and ultimately produced typical cysts like those normally formed in the bowel, though containing more glycogen and somewhat smaller chromatoids than are usually seen. Encystation occurred in this way, under the same conditions, in our cultures of *E. histolytica* from man (Strain D), and in those of "*E. histolytica*" from *M. rhesus* (Strains J and M 4). In these early cultures the amoebae multiplied enormously for the first two or three days, and on the fourth or fifth day numerous cysts, in all stages of development, were generally found. We thus hoped that we had discovered a method of cultivation which would enable us to obtain all stages in the life-cycle at will.

Unfortunately, however, our hopes were not fully realized. We soon found that, after subcultivation in the same media for a variable number of generations, the cysts became fewer and fewer, until the amoebae finally appeared almost to have lost all power of encystation. In the later subcultures they multiplied with the same profusion: but at the end of the reproductive period they merely persisted for a variable length of time, and finally died without forming cysts. Occasionally a few fully-formed cysts would be found: more often abortive attempts at cyst-formation occurred: but abundant crops of normal, mature cysts were never obtained, and for generations often no cysts at all would be discoverable. Even now, our oldest strain of *E. histolytica* (D, isolated in November, 1924, and at the time of writing at the 172nd serial subculture) occasionally forms a few cysts sporadically; but they appear with

no regularity, and we cannot predict whether they will be formed or not in any given culture.

All strains of *E. histolytica* and *E. coli*—whether from man or monkeys—which we have cultivated in the original media of Boeck and Drbohlav, have failed to encyst (in agreement with the findings of these authors). But numerous strains of the first species, when grown in starch-containing media, have encysted more or less plentifully at first, and have then almost ceased to form cysts after a variable number of generations. A few strains of "*E. histolytica*" (from *M. rhesus* and *M. sinicus*) have never at any time encysted in any media—with starch or without; while only one strain of *E. coli* (from man) has ever formed normal cysts, and then only in a few cultures containing starch.

Entamoeba gingivalis has never encysted in any cultures: but this is in agreement with the fact that it never forms cysts—so far as is known at present—in its natural habitat[1]. One of our strains (Σ, from *M. sinicus*) of *Endolimax nana*, however, soon after it was first separated in pure culture, encysted freely and regularly in Boeck-Drbohlav egg-serum medium: but after a number of generations the cysts gradually became fewer, until finally they almost disappeared—just as with *E. histolytica*. All attempts to induce their regular formation once more failed completely. Other strains of this species (from man and monkeys) have formed cysts sparingly, at irregular intervals, or none at all. One strain (Δ 3), for example, was cultivated for 36 days (20 generations) before it was voluntarily given up. During this period over 80 cultures were studied, but in only 3 of these were cysts ever found, and then only in very small numbers. This strain was originally derived from cysts in human faeces.

As we greatly desired—for the purposes of other researches—to be able to obtain cysts (of all species) regularly and plentifully in cultures, we made a long series of experiments in an attempt to ascertain the factors determining cyst-formation. We experimented chiefly with *E. histolytica* (and the closely allied simian form) and *E. coli*, though we have also studied the other species—already mentioned—in this connexion. Unfortunately all our results hitherto have been negative; but as they rule out many possibilities, they seem worth recording briefly for the information of others.

Of the various physical and chemical factors investigated, the following are the chief:

Temperature. In agreement with Boeck and Drbohlav we have found that the optimum temperature for *E. histolytica* (and all other entozoic amoebae studied) is 37°–38° C. Growth will take place at a temperature as high as 41° C.; but we have never been able to cultivate this organism at 30° C. or any lower temperature[2]. When cooled, the amoebae round up, and gradually die. Cutler (1918) states that he cultivated *E. histolytica* at 28°–30° C., but this is not possible with our strains. He also says that encystation was induced

[1] Cf. Dobell (1919), p. 97.

[2] In agreement with Drbohlav (1925 *b*).

by placing cultures in the ice-chest for 2 hours: but since such a procedure always immobilizes the amoebae and causes them to die, it is difficult to believe that it can ever lead to the formation of precystic forms and cysts.

Our experiments indicate that changes of temperature, within the range at which development is possible, have no effect whatever upon encystation.

Hydrogen-ion Concentration. All the strains which we have studied grow best in media which are neutral. Acid and alkali are alike harmful. Variation of *p*H, within the tolerated limits, appears to exercise no influence on cyst-production. Drbohlav (1925 *b*) has made concordant observations.

Tonicity. Our media are made up with Ringer's solution as a diluent, and have a salt-content of 0·94 per cent. But we have found that *E. histolytica* can tolerate considerable changes in tonicity. It will grow in horse-serum or egg media (with starch) even when the usual covering liquid (dilute serum or egg-albumin) is replaced by pure distilled water or double-strength Ringer's fluid. Alteration of the tonicity within these limits has no effect on cyst-production.

Changes in the *Viscosity* of the liquid constituent of the medium appear to have equally little effect. *E. histolytica* and *E. coli* will grow in media composed of inspissated horse-serum (and starch) covered by liquid (inactivated) horse-serum either pure or in any dilution—down to distilled water. They will also grow in similar media in which the liquid consists of a 20 per cent. solution of gum arabic, or any lower concentration.

The *Amount of Liquid* in a culture also appears to have no relation to the induction of cyst-formation. Deep cultures, in which the liquid half fills the tube, often grow quite as well as those in which the surface of the solid is merely moistened by a film of bacteria; but cysts are not formed more freely by deeply submerged organisms than by those which are almost dry. Slow evaporation of the liquid also has no effect (*contra* Chiang, 1925). Similar observations have already been recorded by Drbohlav (1925 *b*).

Oxygen. In ordinary cultures (Boeck-Drbohlav media, and our modifications) *E. histolytica* and all other amoebae studied grow aerobically. Free access of oxygen—such as occurs when the liquid is reduced to a minimum, and the solid is covered by only a damp scum of bacteria and starch—has no inhibitory action upon growth, and does not cause encystation. Nor does the partial anaerobiosis produced by cultivation in tubes containing a considerable depth of liquid. Boeck and Drbohlav found that *E. histolytica* would not grow under completely anaerobic conditions: but it occurred to us that a change from an aerobic to an anaerobic state might favour encystation. Freely growing (aerobic) cultures, however, when transferred to strictly anaerobic conditions, continued to develop but did not form cysts more abundantly than those grown aerobically throughout.

Variations in Salt-Content. All the amoebae which we have tested appear to be little affected by increasing or decreasing—within wide limits—the salt-content of the medium. As already noted, *E. histolytica* will grow in media

(prepared with inspissated horse-serum and starch) in which the solid is covered with distilled water, Ringer's fluid, or double-strength Ringer's fluid. Increase or decrease in the amount of sodium, potassium, magnesium, or calcium, appears to exercise no influence on cyst-formation. The addition of sulphates, chlorides, phosphates, and sodium borate (in tolerated doses), has equally little effect. (We find that magnesium sulphate and sodium phosphate (Na_2HPO_4) are tolerated in considerable concentrations.)

Sulphur, like crystalline sulphates, is without effect in cultures. *E. histolytica* will ingest small particles, but this does not affect encystation. Nor does

Cystine, when added in varying amounts, nor other

Amino-Acids, such as tryptophane, leucine, and tyrosine, which all gave completely negative results. Cutler (1919) found that tyrosine "stimulated multiplication," but we observed no such effect in our cultures.

Sugars, such as dextrose, can usually be added to cultures in very small quantities only, since they are rapidly fermented by the accompanying bacteria with formation of acid. Addition of dextrose, xylose, or arabinose, had no influence on the production of cysts.

Inulin, which we tried originally instead of starch, cannot replace it. It appears to be valueless to *E. histolytica*, and does not influence encystation—as Drbohlav (1925 *b*) has already stated.

Cellulose. Some of the species studied will ingest this substance when present in the intestinal contents. We found that when it was added to cultures of *E. histolytica* the amoebae engulfed the smaller particles: but this did not affect cyst-formation in any way.

Faecal Particles and *Faecal Extracts* (sterilized by boiling), added in various amounts, neither increased nor diminished the number of cysts ultimately produced.

The addition of *Gum Arabic* (dissolved) or *Calcium Pectate* (solid) had equally little effect. *E. histolytica* and *E. coli* will live and multiply in strong solutions of the former, and will ingest particles of the latter—without other visible effects.

Media in which the egg or serum (solid) is replaced by *Agar* will support the amoebae, but as a rule no cysts appear in such cultures.

Fresh *Sterile Tissue* (kidney) added to the medium does not increase the number of cysts formed.

Broth—in any proportion, and whether added to the liquid or incorporated in the solid—in our experience has no effect upon encystation (*contra* Chiang, 1925). It merely favours the growth of bacteria.

Ascitic Fluid (heated or unheated), if used to cover the solid constituent of the medium, accelerates the growth of bacteria, and sometimes gives very rich cultures of amoebae. But it does not induce encystation. The same is true of *Hay Extracts* (aqueous, unheated, and sterilized by filtration) and boiled *Hay Infusion*, both of which can be used in place of dilute serum

or egg-albumin for covering the solid constituent (egg or serum) of the usual media.

The action of various *Alkaloids* on cultures of *E. histolytica* is described elsewhere (Dobell and Laidlaw, 1926). None of those tested influences encystation. Neither do various

Dyes, of which we have tested flavine, eosin, gentian violet, basic fuchsin, neutral red, methylene blue, and malachite green. These substances merely alter the bacterial flora, as also does *Phenol*.

Red Blood-corpuscles, when added to a culture of *E. histolytica*, are speedily ingested by many of the amoebae (as shown by Boeck and Drbohlav): but their presence does not induce encystation. *Coagulated Blood-proteins* are similarly ingested, and are equally without effect on cyst-formation: and inspissated *Whole Blood*, when substituted for serum (solid), gave similar negative results.

We have found that *E. histolytica* from man (or from an experimentally infected cat) is the only amoeba, of those studied, which normally ingests red corpuscles *in vitro*. "*E. histolytica*" from monkeys (isolated directly), *E. coli* (from man), *E. gingivalis*, and *E. nana* (from man or monkey), will not take up red corpuscles when tested under the same conditions.

E. histolytica, however, will ingest very many other solids in cultures, as also will *E. coli*. For example, both species will readily take up yeasts (*Saccharomyces cerevisiae*); and *E. histolytica* will eat and digest the green alga *Pleurococcus*. It will even ingest particles of *Chalk* ($CaCO_3$) and *Calomel* (Hg_2Cl_2), without evident injury. But none of these things appears to influence encystation.

Cutler (1919) stated that skatol (0·5 per cent.), added to his cultures, "rapidly induced cyst formation, practically every one of the amoebae becoming encysted"; but Boeck and Drbohlav (1925) and Drbohlav (1925 *b*) were unable to confirm these observations. Drbohlav (1925 *b*) has also tried adding bile, urine, creatine, nuclein, caseinogen, dextrin, and various other substances—all of which gave negative results.

It occurred to us that the gradual failure to encyst, which we observed in all our strains, might conceivably be due to our having unintentionally selected—by repeated subcultivation of active forms—strains incapable of producing cysts. Possibly all individuals which could form cysts did so, and so dropped out—leaving a non-encysting race. But this supposition has been negatived by numerous experiments. For example, we found that strains originally isolated from cysts do not encyst in cultures any more readily than those started from free forms: while *E. histolytica* recovered from experimentally infected kittens—in which the amoebae never encyst—is still able to form cysts when grown once more in a suitable medium. Moreover, when cysts are formed in a culture, it is possible to continue the strain both by propagating the amoebae which have not encysted, and by hatching the cysts[1].

[1] See next Section.

If this be done—and we have done it repeatedly—the cultures derived from cysts do not show any greater tendency to encyst once more than do those originating from free forms.

As a result of all our work up to the present, therefore, we can only say that *E. histolytica* will often encyst normally in cultures containing solid starch: but it will not do so always, and the number of cysts formed varies from culture to culture and gradually diminishes with continued cultivation. We have found that cyst-production may sometimes be temporarily increased by cultivating the amoebae in starch-free media for one or two generations, and then transferring them to media containing this substance: but the results are uncertain, and the number of cysts produced in any culture cannot be predicted. As regards other factors which determine encystation, in any of the species studied, we are still in complete ignorance.

V. Initiation of Cultures from Cysts.

In the original technique of Boeck and Drbohlav—now successfully practised by many subsequent workers—initial cultures of *E. histolytica* (or other species) are obtained by inoculating culture-tubes with active amoebae from fresh stools[1]; and serial subcultures are then made by the periodic transfer of similar active forms from tube to tube.

But we have found that it is equally possible to initiate cultures from cysts. Under suitable conditions the encysted forms can be made to excyst, and the liberated amoebae can then be cultivated further by the methods already described. In this way actively growing strains of amoebae can be readily obtained both from cysts which have been discharged in the faeces and from those formed in the culture-tube. Since our first successful experiments in this direction (which were made at the beginning of December, 1924) we have started and continued strains of *E. histolytica*, *E. coli*, and *E. nana*—using cysts obtained from men, monkeys, and cultures—on numerous occasions.

The practical importance of this procedure needs no emphasis. It has already enabled us to carry out many experiments—which will be reported fully elsewhere—which could not have been attempted by any other method: and it has also enabled us to study the whole life-cycle of *E. histolytica* and several other amoebae *in vitro* for the first time. But we shall here describe our methods only, without entering into details regarding the results to which they have led.

Cultures can be initiated from cysts as easily as from free amoebae provided that certain precautions are observed. We have found that mature living cysts, of any of the species which we have studied, will hatch and give rise to amoeboid forms once more if they are merely incubated in any of the media already described as suitable for cultivation of the corresponding free amoebae. If

[1] Boeck and Drbohlav (1925), p. 385, report a possible case of cultivation from cysts which "seems reasonable (although it is difficult to prove)." They add that "Other attempts to obtain cultures from encysted forms failed" (*l.c.* p. 386).

favourable conditions are then maintained, the hatched amoebae will multiply and produce normal cultures which can be propagated *ad libitum* by any of the ordinary methods. In other words, the cysts of entozoic amoebae appear to differ in no essential physiological feature from those of the more familiar free-living forms. As is well known, mature cysts of any of the commonly cultivated small free-living amoebae (collectively miscalled "*Amoeba limax*") develop and give rise to new cultures when merely transferred to appropriate fresh culture-medium similar to that in which they were formed. Mature cysts of *E. histolytica*, *E. coli*, and *E. nana* behave in exactly the same way—*mutatis mutandis*—and require no special treatment (with acids, alkalis, digestive ferments, etc.) to enable them to continue their development. We have proved this by repeated experiments.

It is easier to obtain cultures (of all species) from the cysts formed in cultures than from those formed in the intestine and passed in the faeces. We may therefore consider the former first, and will offer an explanation of this difference afterwards.

Initiation of New Cultures from Culture-Cysts. All the species[1] of amoebae studied have formed cysts intermittently *in vitro*. But even in cultures which produce cysts freely we have never observed encystation of *all* the amoebae simultaneously: there is always a maximum production of cysts at a particular time, though both before and after this moment they are always formed by some individuals. For example, with one of our strains of "*E. histolytica*" from *M. rhesus*—Strain J, which produced many cysts at one period in its history—encystation usually occurred as shown in Table II. (It should be noted that, with this strain, the free amoeboid forms seldom survived in cultures for more than 5 or 6 days, and cysts found after the fifth day of incubation were almost invariably dead.)

TABLE II. *Cyst-formation in Cultures of* "E. histolytica."
(Strain J, in Ere + S medium.)

	Age of Culture in Days							
	1	2	3	4	5	6	7	8
Cysts	–	–	+	+ + +	+ +	+	–	–

– = none present. + + = fairly numerous.
+ = few present. + + + = abundant.

Comparable observations have been made on all the other species investigated. With most strains of *E. histolytica* (human and simian) encystation has reached its maximum on the fourth or fifth day; but this is not true of *all* strains. Strain S (from *M. sinicus*), for example, almost always showed the greatest number of cysts on the second or third day. *E. nana* (Strain Σ), which encysted very freely for a time, behaved exactly like the strain illustrated in the table: but *E. coli* (Strain λ, from man) has, upon occasion, produced a good crop of cysts after incubation for only 24 hours.

[1] Excepting, of course, *E. gingivalis*.

When cysts are once formed in cultures they develop rapidly; but if, when fully developed, they are kept at the same temperature (37° C.) they soon degenerate and die. It is extremely difficult to ascertain the exact time taken in development (from the moment when the cyst wall is formed) and also the length of time during which fully-formed cysts can survive at body-temperature. Our experiments indicate, however, that the time occupied in the development of a cyst from the uninucleate to the fully-formed stage (4-nucleate in *E. histolytica* and *E. nana*, 8-nucleate in *E. coli*—all strains from all sources) is approximately 6–8 hours: while the time of survival of fully-formed cysts in cultures kept at 37°–38° C. does not appear to be much longer. It is certain, at any rate, from all our observations, that fully-formed cysts (of any species studied) degenerate and die, and consequently become incapable of any further development, if kept continuously at a temperature of 37° C. for 24 hours. Accordingly, an encysting culture such as that shown in Table II—and this is true of all cultures of all species studied—does not produce an increasing number of cysts with increasing age, but after continued incubation contains nothing but dead cysts in all stages of disintegration.

To prevent degeneration and death of the cysts formed in a culture, they must be cooled to a temperature considerably below that of the body. Ordinary room-temperature (ca. 15°–20° C.) is adequate. As soon as fully-formed cysts are found to be present in sufficient numbers, therefore, the culture should be removed from the incubator and allowed to cool to the temperature of the air. When this is done, all amoebae present in the culture gradually die; all immature cysts cease to develop, and likewise die ultimately; but all fully-formed cysts remain alive and unaltered for a considerable time. Numerous experiments have shown that fully-developed cysts, provided they be cooled, retain their vitality equally well whether they be left in the original culture or transferred to Ringer's solution, physiological saline, tap-water, or even distilled water. Lowering of temperature is the essential factor which determines their survival.

When completely developed cysts are cooled, however, they are not capable of further development immediately. They must be kept cool for a certain time. This time, for *E. histolytica* of man and monkeys, is approximately 1 to 2 days (it varies slightly with different cysts). Thereafter they are able to develop further if transferred to an appropriate culture-medium and incubated at 37° C.: and they remain in a hatchable condition for a definite period of time, which we have determined for *E. histolytica* (both human and simian strains) to be approximately a fortnight.

In order to cultivate *E. histolytica* further from fully-formed cysts produced in cultures, therefore, it is merely necessary to place the culture containing them on the laboratory bench; leave it there for 48 hours; and then, at any time during the next 10 days or so, inoculate a fresh culture-tube from it and put it in the incubator at 37° C. By these simple means a positive result can almost invariably be obtained, and the amoebae which hatch out can

then be propagated by the usual methods. During the last 18 months we have performed this experiment so often, and with so many different strains of all the species studied, that we make this statement with complete confidence. If due attention be paid to all the points just noted, successful cultures are obtainable in 100 per cent. of cases.

Initiation of Cultures from Cysts passed in the Faeces. We have ascertained that cultures can be started, in a similar manner, from cysts (of all species studied) passed in the faeces. Such cysts are capable of hatching in any medium appropriate for the cultivation of the free forms of the species, and of thus originating strains of amoebae which can be propagated *ad libitum*. No special treatment is necessary, beyond that just indicated for culture-cysts: but similar precautions must be taken to insure a successful result. Cysts just passed in the stools do not hatch or develop further if placed immediately in culture-media and incubated at 37° C.: they merely die. They must first be allowed to cool, and must then be kept for a certain time. The time required for "maturation" outside the body, and the length of time during which the cysts remain capable of hatching, are similar to those already stated for culture-cysts.

When cysts of *E. histolytica* (or other species) obtained from stools are treated in this manner, they do not invariably produce good cultures. Many cysts, though apparently mature and normal, do not hatch at all, but degenerate. Others hatch, but the amoebae which they liberate die soon afterwards. The results are less regular and certain than with cysts derived from cultures, and success cannot be obtained in 100 per cent. of experiments.

The difference, in this respect, between culture-cysts and cysts passed in the faeces, appears to be due to the circumstance that the cysts derived from stools are inevitably accompanied by a vast variety of other organisms, and by numerous soluble and fermentable substances (from the faeces) whose presence—or whose products—may be detrimental to the amoebae. On the other hand, cysts from cultures are accompanied by bacteria which are already known—by previous cultivation—to be favourable for the development of the amoebae concerned: and when such cysts are transferred into fresh medium, together with the same concomitant micro-organisms, no new factors are introduced into the system. The equilibrium already established is maintained. But when cysts and faeces are simultaneously inoculated into any culture-medium, an entirely new set of factors—comprising many unknown variables—is introduced, and consequently the result cannot be predicted with certainty.

We have attempted to surmount this difficulty by various expedients; but only one of them, up to the present, has succeeded. The method is as follows:

Cysts in freshly passed faeces are first suspended in water (distilled or tap), physiological saline, or Ringer's fluid. The faeces are thoroughly emulsified in an excess of liquid, and the larger faecal particles removed by filtration

through glass- or cotton-wool. The cysts are then concentrated by sedimentation or spinning in the centrifuge (usually on several successive days). They are not injured by this process, but it kills or removes the majority of unencysted protozoa and *Blastocystis*, while bacteria, yeasts, and soluble substances are greatly reduced in quantity. The cysts—thus concentrated and washed—are now treated with a weak solution of hydrochloric acid, which is allowed to act for a sufficient time to kill off any remaining unencysted protozoa, most *Blastocystis*, and the majority of the bacteria and yeasts. With *E. histolytica* cysts, treated at room-temperature, exposure to a 0·2 per cent. solution of HCl for 2 hours is usually effective. After this treatment the acid is carefully neutralized with a weak solution of soda or sodium bicarbonate (using neutral red as an indicator), and the cysts are then again sedimented or are spun down in the centrifuge. The deposit, containing cysts and faecal débris, is then inoculated into suitable culture-medium and incubated at 37° C.

The only bacteria which usually survive this drastic treatment—when proper precautions are taken (as they should be at every stage) to avoid contamination with unknown extraneous micro-organisms—are the spore-forming intestinal bacilli (whose spores have withstood the acid) and various micrococci and streptococci. Most of these, however, appear to be very unfavourable to the growth of the amoebae; and if the treated material is merely inoculated into a sterile tube of medium and incubated, a poor initial culture is frequently obtained, or a strain which sometimes dies out after one or two subcultures have been made or which can only be propagated with difficulty. To avoid such results, therefore, we now always inoculate treated cysts not into sterile medium but into medium which has previously been sown with bacteria known to be particularly favourable[1] for the amoebae which we desire to cultivate. When this is done, the result is usually—though not invariably—successful.

Cysts of *E. histolytica* (all strains tested) readily withstand 0·2 per cent. of HCl for 2 hours at room-temperature; and cysts have hatched after even 3 hours' exposure, though many were killed in the experiment. *E. coli* cysts appear to be more resistant, *E. nana* cysts more susceptible to the action of the acid; but individual cysts vary in this respect. Resistance is probably directly dependent on the thickness of the cyst wall. On one occasion *E. coli* was successfully cultivated from cysts which had been treated for 1 hour and 20 minutes in *N*/10 HCl (0·36 per cent.) solution. Free amoebae, as a rule, are killed in a few minutes at most; but in one experiment *E. histolytica* was recovered (by cultivation) after treatment of its unencysted forms with 0·2 per cent. HCl for 30 minutes. Culture-cysts, moreover, withstand the acid quite as well as those in faeces; but for ordinary purposes it is unnecessary to subject them to the ordeal, and a greater proportion of cysts hatches if treatment with acid can be omitted.

In carrying out the foregoing technique it is important to use a relatively large volume of 0·2 per cent. HCl solution, in order that sufficient free acid

[1] See next Section, p. 307 *infra*.

may be present. Sometimes when the requisite amount of acid is added to faecal suspensions or the liquid from a culture-tube the resulting mixture gives but a weak reaction with Congo red. To be effective it should give a strong reaction with this dye.

Certain analogies between the results just described and the events which normally occur in the life-cycle of *E. histolytica* and other intestinal amoebae, will at once be evident to readers of the foregoing lines. We are fully alive to these analogies, and their various implications, and have already utilized them in the prosecution of further researches: but we shall not discuss them here, as they are foreign to the present subject. We shall now consider instead another matter which seems to us, as a result of our work, to be of paramount importance in connexion with the cultivation of all entozoic amoebae.

VI. THE INFLUENCE OF THE BACTERIAL FLORA.

"Pure cultures" of *Entamoebae*—such as can be obtained by the Boeck-Drbohlav technique and its various modifications—are never "pure" in the sense that they contain one species of amoeba alone: they inevitably consist of some one species of amoeba accompanied by a diversity of bacteria (and possibly other micro-organisms). But since *E. histolytica* and all other species studied largely depend, in cultures, upon these associated organisms for their food-supply[1], it does not seem feasible to eliminate them entirely.

Strains of amoebae initiated from free forms in faeces are always accompanied by a great many different bacteria derived from the intestinal contents. For the cultivation of many of these bacteria the various media employed are often excellently suited; and consequently they are propagated, along with the amoebae, whenever subcultures are made. But it always happens that the bacterial flora originally present in initial cultures, and early subcultures, undergoes considerable changes—as regards the species and their relative proportions—on continued cultivation. For a variable number of generations the flora fluctuates, though it finally reaches a state of stable equilibrium by a process of natural selection. In this process the amoebae may be exterminated. Accordingly, most freshly isolated strains of amoebae require to be carefully "nursed" for some time before they "settle down" and begin to grow with regularity. But when once a balance has been struck among all the organisms present—and this may require as many as ten or twelve transplantations—the cultures give rise to no further anxiety. Their growth, under constant conditions of medium, temperature, etc., can be foretold with great certainty.

Cultivation of amoebae by methods involving such a number of unknown variables—which sort themselves out, in the end, by chance—is obviously unscientific. Success often depends largely upon luck. It is therefore clearly

[1] Guérin and Pons (1925) state that, in their cultures, *E. histolytica* feeds chiefly upon solid particles of the medium itself—rarely ingesting bacteria. We cannot confirm this observation.

desirable to discover some means of controlling the composition of the bacterial flora at will: but this we have found to be extremely difficult.

That the bacteria in a culture of amoebae exercise a profound influence upon its welfare, nobody who has attempted to cultivate these protozoa can doubt. It is only necessary to have acid-forming bacteria present to convince the most sceptical. Such organisms, by acidifying the medium to a degree which the amoebae cannot tolerate, may wipe out a flourishing culture in a few hours. For example, starch-splitting bacteria, if introduced into cultures containing this carbohydrate, soon make further cultivation of the amoebae impossible: but if they are promptly exterminated (*e.g.*, by the action of flavine), the reaction of the medium is readjusted and the amoebae recover.

It is seldom possible, in our experience, to alter the bacterial flora favourably—or, in fact, to change it appreciably—by merely introducing new species among those already present[1]. Generally, when a new organism is added no effects are observable: it is soon swamped by those already present, unless it be an organism (like some of the accidental contaminants which we have met with) which, by producing acid, or liquefying the solid, affects the whole culture adversely in some indirect manner. Only a few times, out of many experiments, have we succeeded in favourably altering the bacterial flora by introducing new forms among those already established. One such success was with a strain of *E. nana* (Δ 3, isolated from cysts in human faeces). The chief bacteria originally present were spore-forming bacilli, which sporulated freely and quickly in the cultures. The amoebae ingested the spores but appeared unable to digest them, and as a result very poor cultures—requiring transplantation every 24 hours—were obtained. The same medium was then tried, but previously inoculated with a pure culture of a strain of *Bacillus coli* (isolated from the faeces of a *Macacus sinicus*) and incubated for 24 hours before the amoebae were introduced. By repeating this process the new bacteria were able to establish themselves; and thereafter the amoebae, having an adequate food-supply, grew very much better. Richer cultures were obtained, and it was only necessary to make subcultures every second or third day to keep the strain going indefinitely.

We have made many other experiments along similar lines, but the results have almost invariably been disappointing. The only ones which appear worth mentioning are those made in our attempts to induce cyst-formation by such means. We thought it possible that encystation might be primarily dependent—as it undoubtedly is dependent to some extent—upon the bacterial flora, and we therefore introduced the bacteria from a culture of amoebae which had encysted freely into others of a strain which seldom encysted. But the results were completely negative.

The number of different bacteria accompanying any ordinary strain of entozoic amoebae is large, and the task of isolating, identifying, and testing each species individually is colossal. So far we have been able to make only

[1] Drbohlav (1925 *b*) has recorded similar observations.

a few tentative experiments in this direction; but the results, such as they are, appear noteworthy. The method usually adopted has been to plate out and isolate some of the species of bacteria present in flourishing cultures; and then to test their influence upon various species of amoebae by cultivating them from cysts (after treatment with hydrochloric acid, as described in the preceding section) in media previously inoculated with the bacteria whose effects we desired to study. By such methods we have not yet succeeded in obtaining strains consisting of only one species of amoeba accompanied by bacteria of only a single species: but we have been able to get cultures of one species of amoeba in which one known species of bacterium largely predominated.

We have now isolated and tested several different species (or varieties) of bacteria in this way. One of these is the variety of *Bacillus coli* which—as has just been noted—was found to be favourable for *E. nana*[1]. But this *Bacillus* was found not to be favourable for the continued growth of *E. histolytica* (human or simian strains) or *E. coli* (from man). Cysts of these species, when hatched in a medium containing this organism, have always produced poor cultures or strains of amoebae which could be propagated with difficulty or not at all.

From cultures of our Strain D of *E. histolytica* we have isolated, in pure culture, several different other *Bacilli* of the *B. coli* group. Three of these (provisionally named I, III, and IV) have been specially studied. Their chief sugar-reactions and other characters are shown in Table III, the usual symbols (A = acid, G = gas, etc.) being employed. It will be seen that *Bacillus* I has the typical cultural characters of *B. dysenteriae* Shiga[2], while No. III is a typical *B. lactis aerogenes*. No. IV is closely similar to No. III, but differs in being motile, forming less gas, and in giving no Voges-Proskauer reaction. None of these organisms can split starch—solid or in solution.

TABLE III.

	Bacillus I	*Bacillus* III	*Bacillus* IV
Motility	–	–	+
Liquefaction of Gelatin ...	–	–	–
Dextrose	A	AG	AG
Lactose	–	AG	AG
Saccharose	–	AG	AG
Mannite	–	AG	AG
Dulcite	–	–	–
Litmus Milk	Alk. clot	Acid clot	Acid clot
Indol formation	–	–	–
Voges-Proskauer Reaction	–	+	–

These three bacteria, when tested separately, have been found to have very different effects upon the growth of amoebae in cultures. *Bacillus* I, for example, is particularly favourable for the cultivation of *E. histolytica* (all

[1] This experiment was confirmed by hatching the cysts of this species among these bacilli, and propagating the amoebae further in the same environment.

[2] It is inagglutinable by M.R.C. standard specific antiserum for this species, however, and is therefore not a true Shiga's bacillus.

strains tested—mostly simian) and *E. coli* (from man). In the presence of this bacillus, in predominating numbers, the cysts of these species hatch readily: the hatched amoebae then ingest the bacilli freely: and cultures so started can usually be propagated with ease. With *E. nana*, however, the results are very different. *Bacillus* I appears quite unsuited to the needs of this amoeba, which does not readily ingest it and soon dies out when it is the predominant bacterium. But with *Bacillus* IV the reverse is the case. This organism is very favourable for the cultivation of *E. nana*, but unfavourable for *E. histolytica*. Finally, *Bacillus* III appears to be definitely detrimental to all the amoebae tested. Successful cultures have never been obtained in media in which it predominates.

The different influences exercised by these three bacilli are well illustrated in the following experiment: A mixture of cysts consisting of many of "*E. histolytica*" and a smaller number of *E. nana* (from the faeces of *M. sinicus*), was concentrated, carefully washed, and treated with hydrochloric acid (0·2 per cent. for 2 hours) as described in the preceding section. The mixed cysts, after neutralization, were then inoculated into 3 tubes of medium (HSre + S)—one containing a pure culture of *Bacillus* I, another pure *Bacillus* III, and the third pure *Bacillus* IV (all incubated for 24 hours previously). The amoebic inoculum was thus the same in each tube, but the bacterial flora different. On incubation and subcultivation these cultures ultimately gave the following results: with *Bacillus* I, a pure culture of "*E. histolytica*"; with *Bacillus* III, no amoebae of either species; with *Bacillus* IV, a pure culture of *E. nana*. The last result is, perhaps, the most striking, as in the inoculum there were far more cysts of *E. histolytica* than of *E. nana*—which, in the end, alone was able to establish itself in the presence of *Bacillus* IV.

Many other similar experiments have been made, and all of them emphasize the importance of the part played by the bacterial flora in cultures of entozoic amoebae. But as our work along such lines is still in progress, and our results are therefore not yet definitive, we must content ourselves for the present with the few foregoing somewhat desultory remarks upon this subject.

VII. The Isolation of "Pure" Cultures.

Whenever cultures of entozoic amoebae are required for experimental purposes, it is first necessary to make sure that they are "pure" (*i.e.*, contain only one species of amoeba together with various—and usually indeterminate—species of bacteria). But this is often a matter of no little difficulty. Multiple infections are very common, both in men and in monkeys; and the media generally employed are suitable for the cultivation of many amoebae and flagellates. Consequently, when cultures are initiated from amoebae or cysts in faeces, the organisms eventually isolated are frequently found to consist of a mixture of species. If pure cultures are desired, therefore, these species must be separated.

The *bête noire* of would-be cultivators of intestinal amoebae is the fungus *Blastocystis*, which is extremely common in the faeces of men and monkeys—not to mention other animals—and grows freely in nearly all media suitable for the cultivation of entozoic protozoa. Its presence in a culture is rapidly fatal to amoebae and flagellates, and in our experience it is impossible to maintain any strain of amoebae satisfactorily *in vitro* in its company. Unless it is eradicated completely at an early stage, it eventually overgrows and kills all protozoa—usually within a few generations[1]. In view of its great practical importance, therefore, we shall first say a few words about

The Eradication of Blastocystis.—We have made many attempts to discover some substance which, when added to the culture-medium, would kill *Blastocystis* but leave the amoebae unharmed. But it is a most resistant organism, and has always withstood far more drastic treatment with dyes, acid, antiseptics, alkaloids, etc., than any of the amoebae studied: while it remains apparently unaffected, for long periods, by changes of temperature and other conditions which are fatal to most intestinal protozoa. For example, *Blastocystis* will continue to flourish in medium containing a concentration of flavine which rapidly kills all amoebae. It will survive in distilled water, at room-temperature, for at least 3 weeks—about 24 hours usually being the maximum for *E. histolytica* under the same conditions: and it has resisted treatment with 0·2 per cent. hydrochloric acid for 3 hours, and even with 0·36 per cent. for 1 hour and 20 minutes. (All amoebae are usually killed by 0·2 per cent. HCl in a few minutes.) Moreover, it can survive in incubated cultures (37° C.) much longer than any amoebae which may be associated with it, and in solid stools at room-temperature for at least a fortnight. It flourishes in nearly all modifications of Boeck-Drbohlav media which we have tried, and even grows abundantly—as Barret (1921) has shown—in so simple a medium as dilute serum. On the whole, therefore, it is an extremely difficult organism to deal with.

At the very beginning of our work, however, we discovered that media containing solid rice-starch—prepared as described in a previous section—are highly unfavourable to *Blastocystis*. This is one of the great advantages of such media. If a strain of *E. histolytica*, contaminated—however heavily—with *Blastocystis*, is transferred into starch-medium and cultivated in it for several successive generations, the amoebae survive, but the *Blastocystis* is rapidly exterminated. With *E. histolytica*[2] this procedure hardly ever fails. It is only unsuccessful, in our experience, when starch-fermenting bacteria happen to be also present: and in such cases the addition of flavine to the cultures (as already described) will usually lead to the desired result. Provided

[1] We are aware that Thomson and Robertson (1925) state that they were able to cultivate *E. coli* with *Blastocystis* for 69 days. But this is surely a most exceptional experience; and the same writers also say that "this culture, as were all the others, with the exception of the *E. histolytica*, was badly contaminated by *Blastocystis hominis*, which grew and multiplied at such a speed as to completely occlude all the protozoa present."

[2] Most of our experiments have been made with "*E. histolytica*" from monkeys.

that the starch is not split, cultivation in media containing this substance is a certain method of eradicating *Blastocystis*.

Blastocystis is sometimes just as troublesome in cultures initiated from cysts as in those prepared from active amoebae in fresh faeces. Even when the cysts are concentrated and washed many times, and treated with acid for 2 or 3 hours, isolated individuals may survive: and consequently, when cultures are then made and incubated, it makes its unwelcome appearance once more along with the amoebae—though sometimes it does not show itself before 2 or 3 subcultures have been made. When it does so, continued cultivation in starch-medium is almost always an effective remedy.

During our experiments with various bacteria—described in the preceding section—we also discovered, accidentally, that the presence of "*Bacillus* I" in large numbers in a culture is highly detrimental to *Blastocystis*. If cysts of *E. histolytica* are hatched in medium containing this bacillus, any *Blastocystis* which happen to accompany them are usually exterminated in the initial culture. They may begin to grow, but they then rapidly degenerate and die. Consequently, cultures of *E. histolytica* entirely free from *Blastocystis* can usually be obtained rapidly and certainly by hatching the cysts of this amoeba in medium containing solid rice-starch and a good growth of "*Bacillus* I." This method hardly ever fails, and has been used repeatedly by one of us (C. D.) for isolating strains of "*E. histolytica*" from monkeys, which are invariably, in our experience, heavily infected with *Blastocystis*.

Drbohlav (1925 *e*), after failing by all other methods to eliminate *Blastocystis* from his cultures, succeeded in doing so (with *E. coli*) by adding 1 per cent. of dextrin[1] to his medium. But this procedure seems to be less certain than ours, and to have little practical utility.

Separation of Various Species of Amoebae present in Mixed Cultures.—It seldom happens that a single species of amoeba is present in cultures made from the faeces of men or *Macacus* monkeys[2], for mixed infections are common. Even when only one species can be found in a stool by microscopic examination, cultivation may reveal the presence of others. To separate these from one another in pure culture is often difficult; but we have frequently succeeded in doing so, and will therefore note very briefly some of the methods employed.

E. coli and *E. histolytica* can be separated by inoculating a mixed culture into a kitten. If the animal becomes infected, *E. histolytica* can then be recovered in pure culture from its dysenteric discharges—since *E. coli* cannot infect kittens. *E. coli* could be obtained in pure culture from the original mixture by transplanting it into medium containing emetine, which is specifically poisonous to *E. histolytica* but comparatively harmless to *E. coli* (Dobell and Laidlaw, 1926). We have not performed this experiment, but

[1] "Dextrin" is a substance of such ill-defined chemical composition that constant results can hardly be expected from its employment.

[2] Every monkey which we have yet studied carefully has been infected with at least two species of intestinal amoeba, and the majority have harboured three or four

we are certain that it would succeed: and we have actually obtained a pure culture of *E. nana* by this method from a mixed culture of this species and *E. histolytica*.

A mixed infection with *E. histolytica* and *E. nana* can also be separated into its two constituents by cultivating from cysts in a bacterial environment appropriate to either one or the other species. (A successful experiment of this sort has already been described on p. 310.) Moreover, in a mixed culture of amoebae, only one species may encyst at a given moment. When this occurs it affords a ready means of obtaining a pure culture of the encysted species—whichever it be. It is only necessary to treat the mixture of cysts and amoebae with acid (as already described), in order to kill off all free amoebae and so obtain cysts—of a single species—which can be used to initiate a pure culture by the methods previously described. This procedure has been successful, with various species, on many occasions.

Again, the time of survival of different species, both in incubated cultures and outside the body, often varies considerably, and use can be made of this to obtain pure cultures. In a given culture, for example, in which two species of amoebae are present, one may live for only two or three days' incubation, the other for as long as a week. By prolonged incubation, therefore, and subcultivation, the longer-living species can be obtained pure. Furthermore, some species (*e.g.*, *E. coli*) are very sensitive to cold, while others (such as *E. histolytica*) are far less easily killed by a lowered temperature. Consequently, if a mixed culture be removed from the incubator and kept at room-temperature for a certain time, one species often survives longer than the other: and by making subcultures after various intervals of time, the more resistant species can finally be isolated in pure culture. We have succeeded many times in obtaining pure cultures by this method, and have also used it with success for the separation of various intestinal flagellates.

Advantage can also be taken of the fact that certain media are far more suitable for the growth of some species than they are for others. Thus, medium composed of horse-serum, Ringer-eggwhite, and starch, is excellent for the cultivation of *E. histolytica* but unfavourable for *E. nana*. By continued subcultivation of such a mixture of species in this medium we have ultimately obtained *E. histolytica* in pure culture on several occasions.

Various other procedures can obviously be tried, and many hints in this connexion will be found in the three preceding sections of this paper. We have had considerable practice in isolating pure cultures during the last 18 months; and though we have often succeeded, we have also often failed, and some species we have never yet been able to cultivate alone. We do not claim, therefore, that the methods indicated are infallible for all strains, or all species, but offer them rather as helpful suggestions to others who may be engaged in similar work.

VIII. Effects of Cultivation on the Infectivity of *E. histolytica*.

Boeck and Drbohlav (1925) and Drbohlav (1925 *b*) found that when *E. histolytica* is cultivated in their media—even for a prolonged period—it does not lose its virulence or power of infecting kittens. They have reported a successful infection (accompanied by acute amoebic dysentery) produced by intrarectal inoculation of amoebae which had been cultivated for about 5 months since isolation[1].

In order to ascertain whether the very rich cultures of *E. histolytica* which we obtained in our media containing starch had also remained virulent and infective, we carried out some similar experiments with kittens. All these trials were made with Strain D, which had been previously shown by Drbohlav (1925 *b*) to be infective at the eighth subculture when grown in Boeck-Drbohlav medium. Our first experiments were made with amoebae which had been cultivated continuously in starch-containing media for 13 serial subcultures (38 days). Four kittens (Nos. 4, 5, 6, 11) were inoculated intrarectally, with all due precautions and using rich cultures, but none of them became infected. The results were completely negative.

Similar experiments were then made with the amoebae present in 33rd, 36th, and 46th serial subcultures; but these likewise gave entirely negative results. Although we inoculated 7 kittens intrarectally (one of them twice) with cultures containing enormous numbers of active and apparently normal amoebae—in numbers and freshness far surpassing those that can ever be obtained from dysenteric stools—we did not succeed in infecting a single kitten.

All these experiments were conducted with extreme care. The technique adopted was one which almost invariably gave positive results with amoebae known to be infective; and the inoculum was retained in every experiment with one exception, when a portion of it was evacuated within ten minutes. One kitten received two injections, the others one apiece. After inoculation every kitten was seen daily, at frequent intervals; and every specimen of faeces passed was inspected, and many were examined microscopically. When bloody mucus appeared in the evacuations—as happened occasionally[2]—it was always examined with the microscope, and from time to time culture-tubes were inoculated from suspicious discharges. Two kittens were killed after a sufficient time had elapsed for the establishment of infection, but no amoebae or amoebic lesions could be found on careful *post mortem* examination. One kitten died as a result of a natural infection with intestinal worms, and another was killed in the last stages of pneumonia. Five of the seven kittens remained normal and healthy, however, so that in these the experiments were

[1] See Boeck and Drbohlav (1925), experiment with Kitten 120, p. 403. The generation of the culture used is stated (p. 389) to have been the 85th.

[2] This has frequently been observed also in normal (uninoculated) kittens, and is not—in itself—evidence of amoebic infection.

not complicated by intercurrent infection. From every kitten conclusive evidence of non-infection was ultimately obtained.

The last experiment performed (with Kitten No. 13) differed from the others in the following particulars. Since earlier results had shown that starch-fed amoebae failed to infect, the amoebae—from starch-cultures—were cultivated for 25 days (14 subcultures) in starch-free medium to which blood was added. (The medium was HSre supplemented by 1 or 2 drops of fresh human finger-blood.) Very poor growth was obtained in this: and after the first few cultures the amoebae—to our surprise—refused to ingest the red blood-corpuscles. Thereafter the cultures were only maintained with great difficulty, and usually had to be transplanted every second day—sometimes every 24 hours. The 46th subculture, which was ultimately injected into Kitten 13, was not rich: but when it was inoculated, at the same time, into medium containing starch, it yielded an abundant and normal growth.

As this strain of *E. histolytica* was originally isolated from a case of acute human amoebic dysentery, and as it had readily infected a kitten at an earlier stage in its cultural history, we are forced to conclude that cultivation in starch-containing media led to complete loss of virulence and infectivity for kittens. Our experiments were not numerous; but they were all so carefully conducted, and their results so consistently negative, that they have convinced us that this strain is no longer infective.

It may be of interest to add that our other human strain (F) of *E. histolytica*, which was isolated in Boeck-Drbohlav medium, promptly infected a kitten when tested a few days later (1st subculture). This strain, moreover, was cultivated—as already noted—from the stool of a patient who was not suffering, and had never suffered, from amoebic dysentery.

It is remarkable that Strain D, which apparently lost its infectivity as early as the 26th cultural generation, has never lost its power of ingesting red blood-corpuscles. From time to time it has been tested by adding a drop of fresh finger-blood to a culture and examining the amoebae shortly afterwards, when it has invariably been found that many of them have taken up corpuscles in large numbers. The strain was last tested in this way at the 165th subculture—17 months after isolation—and was found to be still as avid of corpuscles as at earlier generations.

It is clear from these observations, and many similar ones which we have made on other strains of amoebae, that ability to ingest red blood-corpuscles is in no way correlated with ability to infect and produce dysentery in kittens.

Our experiments have therefore shown that cultivation of *E. histolytica* in starch-containing media leads—at least in some strains—to complete loss of infectivity, virulence, and pathogenicity for kittens; though such cultivation does not in any way cause the amoebae to lose their taste for human red blood-corpuscles even after a prolonged period (at least 17 months).

As all our animal experiments[1] with Strain D gave entirely negative results,

[1] All these experiments were performed by one of us (C. D.), but the results were seen by both.

they need not be described in detail: but full records have been kept, and the essential facts—in support of the foregoing statements—are briefly abstracted in Table IV.

TABLE IV.

Kitten No.	Date of inoculation	Serial No. of Subculture	Age of Subcult. in days	No. of days since amoebae were isolated	Result
4	8. i. 25	26	2	66	Negative. Discharged as normal 9. ii. 25.
5	8. i. 25	26	2	66	Negative. Killed and examined 24. i. 25. Normal: no amoebae or amoebic lesions found.
6	8. i. 25	26	2	66	Negative. Found dead 25. i. 25. No amoebae or amoebic lesions. Enteritis and peritonitis, due to heavy infection with *Ascaris* and cestodes.
10	23. i. 25	33	4	81	Negative. Discharged as normal 4. iii. 25.
11	8. i. 25	26	2	66	Negative. Normal on 29. i. 25.
	29. i. 25	36	2	87	Negative. Killed 6. ii. 25. Normal: no amoebae or amoebic lesions found.
12	29. i. 25	36	2	87	Negative. Discharged as normal 26. ii. 25.
13	13. ii. 25	46	2	102	Negative. Very ill (severe coryza) 9. iii. 25: killed. No amoebae or amoebic lesions found. Pneumonia in both lungs.

IX. SUMMARY.

The method devised by Boeck and Drbohlav for the cultivation of *E. histolytica* has been successfully used for cultivating this species and various other entozoic amoebae of man and monkeys.

Various modifications of the original technique have been tried, and several improvements are here described. Among these are methods of cultivation (involving the addition of solid rice-starch to the medium) whereby (1) more luxuriant and prolonged growth of the amoebae, and (2) all stages—including encystation and excystation—in the life-cycle of *E. histolytica* and other species can be obtained *in vitro*.

Methods by which cultures of entozoic amoebae can be initiated from encysted forms have been discovered and are also described, and some indications are given for the isolation of pure strains from mixed cultures.

The influence of the accompanying bacterial flora upon the various amoebae cultivated has been studied, and is briefly discussed in the light of certain experimental findings.

Loss of infectivity to kittens has been observed in *E. histolytica* as an apparent result of cultivation in media containing solid starch.

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—— (1925 *c*). Culture d'*Entamoeba dysenteriae* type *tetragena minuta*. *Ibid.* p. 358.

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ADDENDUM (18. vi. 1926).

Since the foregoing pages went to press the three following relevant papers have come to our notice:

Howitt, B. F. (1925). The cultivation of Endamoeba gingivalis (Gros). *Univ. California Publ. Zool.* xxviii. 65.

Kofoid, C. A. and Wagener, E. H. (1925). The behaviour of Endamoeba dysenteriae in mixed cultures with bacteria. *Ibid.* p. 127.

—— —— (1925*a*). Studies of the effects of certain drugs upon Endamoeba dysenteriae in vitro. *Ibid.* p. 155.

Although these papers are stated to have been issued on November 24, 1925, they did not reach our Library (from the University of California Press) until June of the present year. We regret, therefore, that we have been unable to consider their contents in the present paper or in that which we published in the preceding number of *Parasitology*.

C. D. P. P. L.

A COMPARATIVE STUDY OF THE STRUCTURE OF THE HEAD AND MOUTH PARTS IN THE HIPPOBOSCIDAE (DIPTERA PUPIPARA).

By B. JOBLING.

(*From the Wellcome Bureau of Scientific Research, London.*)

(With Plates XI–XV and 4 Text-figures.)

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Introduction.

Though the structure of the head and the proboscis of Hippoboscidae has been studied by many observers, their descriptions are in some respects incomplete. When, therefore, living specimens of *Lynchia maura* Bigot were brought to the Wellcome Bureau by Dr W. N. F. Woodland from Algiers and these were successfully bred on pigeons in captivity, I availed myself of the opportunity to reinvestigate their structure. In addition, *Melophagus ovinus* L. and many other forms belonging to the family were examined with special reference to the structure of the head and mouth parts.

Before giving an account of the observations I have made I wish to take this opportunity of thanking Dr C. M. Wenyon for placing at my disposal the living specimens of *Lynchia*, as also Dr Edmund Sergent, Director of the Institut Pasteur, Algiers, and Dr M. Béguet of the same Institute for preserved specimens of these flies. My thanks are also due to Mr F. W. Edwards, who kindly permitted me to examine Hippoboscidae in the collection at the

British Museum (Natural History Department); to Major E. E. Austen and to Dr K. Jordan for some useful suggestions; to Mr T. Dalling of the Wellcome Physiological Research Laboratories for constantly supplying me with living *M. ovinus*; and to Miss I. M. Bellis for assistance given in connection with the literature.

Methods.

The chitinous parts were studied both in freshly killed flies as well as in specimens preserved in 70 per cent. alcohol. The flies were either soaked for twenty-four hours or boiled for about five minutes in 10 per cent. solution of caustic potash. For structures such as the antennae and the distal parts of the proboscis the cold method was found to give much better results than boiling. During the investigation it was noted that the structure of the membranous and non-pigmented parts becomes invisible in clove oil. On this account all structures were examined both in canada balsam and in 70 per cent. alcohol. Dissections were carried out in clove oil, as in this medium there is no danger of losing the small parts. The muscles of the rostrum were studied by dissection under the binocular microscope and in longitudinal sections (25–30 μ) of the head. For muscles of the haustellum transverse sections were used. As longitudinal sections of the bulbous part of the haustellum did not give satisfactory results, the procedure was adopted of cutting only half the bulb on the microtome, the remaining half, stained and mounted in canada balsam, being used for *toto*-examination. For fixation Bouin's picro-formol, with the addition of one or two drops of chloroform, was used. For serial sections double embedding (photoxylin followed by paraffin) proved to be more efficient than the ordinary paraffin method. The sections were for the most part stained with Mayer's acid haemalum and counter-stained with an alcoholic solution of eosin.

The Integument.

The chitinous integument of the Hippoboscidae consists of two layers: the outer or primary cuticula, and the inner or secondary cuticula. The primary cuticula is homogeneous, apart from the presence of pigment, most of which lies next the inner surface (Pl. XIV, fig. 39; Pl. XV, fig. 53), and is very hard and brittle. The inner or secondary cuticula, which overlies the hypodermic layer, is also usually more or less homogeneous, is devoid of pigment and is relatively very soft. Eosin stains this layer a slightly reddish colour and picric nigrosin gives a blue stain. The primary cuticula is hardly stainable at all. These two layers are not equal in thickness, the primary cuticula being thinner than the secondary, which latter is specially thick in some places. In all the membranous parts the primary cuticula is extremely thin, while the secondary cuticula is replaced by a very thick elastic layer which exhibits a fibrous structure and is stained a grey-blue colour by haemalum. It may also be mentioned here that the chitinous lining or intima of the salivary ducts (Pl. XIV, fig. 38) stains similarly to the elastic modification of the secondary cuticula

just mentioned but is homogeneous. In preparations soaked in caustic potash the intima is quite transparent and colourless, while in the cleared preparations it is difficult to detect.

If the head of these flies be kept for some time in caustic potash it is possible to remove all the primary cuticula from the head capsule. The same may be done with the thorax. The setae, hairs and spines are composed entirely of primary cuticula. In the sclerites of the mouth parts I could not find any subdivision of the integment into primary and secondary layers, its entire microscopic texture closely resembling that of the primary cuticula of the ordinary integument.

THE HEAD CAPSULE OF *LYNCHIA MAURA* BIGOT.

(Plate XI.)

The dorsal surface of the head of *Lynchia maura* is flattened and almost circular in outline. The projecting frontal part, called the fronto-clypeus (*F.C.*), is, according to Peterson (1916), formed by the fusion of two unpaired sclerites —the front and the clypeus. The anterior border of the fronto-clypeus is strongly incurved and there is a narrow furrow stretching across the fronto-clypeus lying between the dorsal prolongations of the first antennal segments (1 *S.*), this furrow being very distinct in longitudinal sections of the head. The lateral parts of the fronto-clypeus invade the cavity of the head capsule, producing posteriorly two very deep and darkly pigmented pits, the antennal pits (*A.P.*), the posterior and dorsal parts of which are formed by the first antennal segment. The first antennal segment is completely fused with the sides of the antennal pit and the line of fusion is only seen in cleared specimens. The posterior border of the fronto-clypeus is separated from the rest of the dorsal surface of the head—the vertex (*V.*)—by the frontal or ptilinal suture (*Pt.S.*).

In a few generalised Diptera only "the stem of the epicranial suture is present and marks the line of fusion of the two halves of the vertex" (Peterson, 1916). In the specialised Diptera this region may be subdivided into distinct areas, since they differ somewhat in structure. In *L. maura* the vertex has the same structure as in the Muscidae: the only difference being that in this fly it is broadened between the compound eyes (*E.*) and much flattened. This condition is found in all the Hippoboscidae.

The median part of the vertex is the mediovertex (*M.*). It is sharply distinguished from the other parts. In the Muscidae this part has been termed by Townsend (1908) "frontalia." The anterior border of the mediovertex has the same breadth as the ptilinal suture, though posteriorly this part becomes more narrow where its two parallel sides lie between the compound eyes. It extends to the posterior third of the vertex where it gives off two extensions which terminate at the vertical bristles (*V.B.*). The mediovertex has the same structure as the ptilinum (Pl. XI, fig. 9) and, like the latter, is composed of a thick, laminated membrane covered with highly pigmented scales (Pl. XI,

figs. 4 and 5). On account of these scales the surface of the mediovertex is rough and dark in colour.

The ptilinum (Pl. XI, fig. 9) has the shape of a triangular sac and is concealed in the head capsule in all adult flies. Its anterior wall is attached along the posterior border of the fronto-clypeus, whereas the posterior wall is continuous with the mediovertex. The scales of the ptilinum are in structure essentially similar to the scales of the mediovertex, and the transition from the narrow scales of the ptilinum to the broad scales of the mediovertex can be traced along the posterior border of the ptilinal suture. When the fly is hatched the inflated ptilinal sac pushes off the anterior part of the pupal case and the hatched fly appears with a greatly swollen dorsal surface of the head. It can then be seen that the inflated ptilinum forms a continuous surface with the mediovertex. In a very short time after hatching the ptilinum becomes retracted into the head capsule, while the mediovertex becomes narrower and acquires a wrinkled surface. On the basis of the preceding description of the ptilinum and the mediovertex the author regards them as morphologically equivalent.

The posterior part of the vertex, limited in front by the mediovertex, is represented by the vertical triangle (*V.Tr.*) or "postvertex," as it would be better to term it. It is highly chitinised and occupies the same position as in the Muscidae. In *Ornithomyia*, *Ornithoeca*, *Ornitheza*, *Ornithophila*, *Ornithoctona*, *Ornithopertha*, *Stenopteryx* and *Lipoptena* the vertical triangle has a darkly pigmented triangular spot which contains the ocelli.

The lateral parts of the vertex which lie between the compound eyes and the mediovertex are strongly chitinised and covered with very fine setae. Moreover, each of these regions has a very long and thick seta or vertical bristle (*V.B.*) at the posterior border, and two other setae, one of which is situated in front of the anterior border of the compound eye and the other at the side of the mediovertex. Lowne (1890–2) named these parts the poracephala. Hewitt (1914) in his description of the head capsule of *Musca domestica* called them genae. The genae (*G.*) occupy comparatively small areas on the head of *L. maura* and they are not separated by a suture from the other regions of the head. Posteriorly the genae are bordered by the compound eyes, and in front are separated from the fronto-clypeus by the ptilinal suture, surrounding the aperture through which the proboscis is pushed laterally, their anterior parts bearing highly chitinised setae.

The ventral surface of the head (Pl. XI, fig. 2) is slightly convex and piriform in shape, with the compound eyes projecting laterally. The parts lying anteriorly to the occipital foramen and between the compound eyes are the postgenae (*Pg.*). They are covered with short, fine setae and, as in all other Diptera, are not separated from the other regions by sutures. The median portions of the postgenae are membranous. In those species of Hippoboscidae which are parasites of birds this membrane occupies a very small space, extending from the membranous flap (*Fl.*) to the projecting and

greatly thickened postero-median part of the postgenae, but in those parasitic on mammals this membrane occupies nearly all the ventral surface of the head. The transition of the postgenae into the membrane is apparently due to the great retractability of the mouth parts.

The posterior surface of the head is concave and triangular in outline (Pl. XI, fig. 3), the occipital foramen (*Oc.F.*) occupying the median and ventral portion of this surface. The region which is limited by the posterior border of the vertex and which surrounds the occipital foramen on each side is the occiput (*Oc.*). The chitinous wall of the occiput has very thick layers of primary and secondary cuticula, consequently the dorso-lateral thickenings (Peterson, 1916) are hardly distinguishable. The highly pigmented stripes extending from the posterior corners of the vertical triangle and broadly connected in the middle of the occiput are probably traces of these thickenings.

The occipital foramen (*Oc.F.*) is arched dorsally and ventrally, the dorsal arch being narrow and the ventral relatively broad. At the points where the dorsal and ventral arches meet there are two occipital condyles (*Oc.C.*). To each of them is attached a latero-cervical sclerite (*Lc.*) known as the neck or jugular sclerite. The cervical membrane is very thick and is attached to the borders of the occipital foramen. In the head of Muscidae there is a funnel-shaped pit projecting into the head capsule on each side of the occipital foramen. From these projections the tentorial thickenings, which extend along the postgenae, are given off. In *L. maura* these projections are very distinct, but the tentorial thickenings are invisible, probably being incorporated with the thick postero-median part of the postgenae.

The body of the tentorium (Peterson, 1916) or jugum (of other authors) is present. It is composed of two very small fork-shaped sclerites which occupy the same position as in the Muscidae and are connected with each other by their membranous ends in front of the occipital foramen in the head capsule. They serve for the support of the cephalic ganglion.

The infolding of the ocular sclerite produces a very distinct ring-shaped thickening (*O.S.*) projecting into the head capsule. From its anterior part arise the postgenal thickenings (*Pg.Th.*) which spread along the postgenae. All the internal thickenings of the head are composed entirely of secondary cuticula, the primary cuticula in these regions remaining thin and distinguished by their pigmentation.

THE HEAD CAPSULE OF *MELOPHAGUS OVINUS* L.
(Plate XII.)

In accordance with the view that *M. ovinus* is the most highly specialised fly amongst the Hippoboscidae the shape of its head capsule is to be regarded as having undergone much greater modification than that of *L. maura*. All the surfaces of the head of this fly have a triangular shape (Pl. XII, figs. 10, 11 and 13). The frontal margin of the fronto-clypeus (*F.C.*) is semi-circular; the surface of the sclerite is smooth and almost flat, and a very narrow and

faint median furrow extends from the anterior margin to the middle. The fronto-clypeus is separated from the vertex (*V.*) and genae (*G.*) by the arched ptilinal suture (*Pt.S.*), the lateral ends of which are bent towards the ventral surface of the head (Pl. XII, fig. 11). The fronto-clypeus is invaginated into the head capsule, producing the antennal pits (*A.P.*), which in this fly are much better developed than in *L. maura* and have very distinct margins in the anterior parts. The first antennal segments (1 *S.*) are, as in *L. maura*, fused with the anterior sides of the antennal pits.

The vertex (*V.*) consists of regions similar to those of *L. maura*; it is, however, much broadened laterally. The mediovertex (*M.*) is greatly reduced and is provided with extremely small scales (Pl. XII, fig. 14) with much broader spaces between them than on the membrane of the ptilinum, where the scales (Pl. XII, fig. 15) are very strongly developed and are much darker. On the posterior margin of the ptilinal suture the transition of the ptilinal scales into the scales of the mediovertex is very gradual.

The ptilinum (*Pt.*) is crescent-shaped and has the same attachment as in *L. maura*, the only difference being that its lateral ends are bent towards the ventral surface of the head.

The vertical triangle (*V.Tr.*) is distinctly separated from the occiput and its posterior margin is very thickened and projected into the head capsule. In the drawing this thickening is shown by a dotted line. The lateral parts of the vertex, which occupy a position between the mediovertex and the suture of the ocular sclerites, are triangular in shape and covered with very stout setae. As a rule these setae are much more strongly developed in the female than in the male. This difference was noted by Ferris and Cole (1922). On each of the posterior corners of this region there is a vertical bristle (*V.B.*). The compound eyes (*E.*) are greatly reduced; they are oblong and their anterior regions extend ventrally. I have found about 135 ommatidia in each compound eye. The ocular sclerite (*O.S.*), as in *L. maura*, produces a thickening on the inner side of the head capsule and its posterior parts are fused together. It is obvious that this sclerite has been modified in consequence of the reduction of the compound eye.

The genae (*G.*) in this fly occupy very small spaces between the anterior borders of the compound eyes and the ptilinal suture. As the result of the reduction of the compound eyes and the greater flattening of the dorsal surface of the head, the lateral sides of the postgenae (*Pg.*) extend on to the dorsal surface and their lateral margins are slightly membranous and carry each a row of strong setae. The ventral surface of the head is occupied by a median membranous region representing the modified postgenae, the lateral surface of which is crumpled and covered with short, stout setae. The median membranous part of the head of *M. ovinus* is more highly developed than in all the other species of the Hippoboscidae. It has a row of long setae along the anterior border on both sides of the opening through which the proboscis is pushed from the head. Moreover, there are some very short setae in the

latero-anterior parts of the membrane and two very long ones situated on small chitinised areas. These areas are the remnants of the postgenae, their number and shape varying in different individuals.

The occiput (*Oc.*) occupies the same position as in *L. maura*. The position of the occipital foramen (*Oc.F.*) is median and slightly dorsal. It is much broader than in *L. maura* and is also composed of a dorsal and a ventral arch. The occipital condyles (*Oc.C.*) are much more distinct and are sharply delimited from the latero-cervical sclerites (*Lc.*). On each side of the dorsal arch of the occipital foramen there is a very shallow and broad pit homologous with the funnel-shaped pit of *L. maura*. From the ventral parts of these pits are given off thickenings on the inner surface of the postgenae, which are apparently the remnants of the tentorial thickenings. These thickenings are shown in the drawing by dotted lines. The dorso-ventral thickenings are indistinct. I was unable to detect the body of the tentorium. Probably in this species it has disappeared or is composed of such small sclerites that they were easily torn off with the trachea during the dissection of the head capsule. The chitinous integument is not so hard as it is in *L. maura*, and the primary cuticula is much more lightly pigmented. The occiput and the postero-median part of the postgenae are the thickest parts in the head capsule.

THE ANTENNAE. (Pls. XI and XII.)

In the different works dealing with the antennae of the Hippoboscidae the authors do not agree regarding the number and the position of the antennal segments. Lyonet (1829) described the antennae of *M. ovinus* as being composed of three segments, the antennal pit representing the first segment. According to Dufour (1845) the antennae consist of one immobile segment. Müggenburg (1892) came to the conclusion that the antennae of *M. ovinus* have only two segments. The correct number of the antennal segments in the Hippoboscidae was established by Massonnat (1909). This author showed that the first antennal segment is fused with the sides of the antennal pit but as he studied the structure of the antennae in sections his description of the structure of the segments is necessarily incomplete. For a correct morphological description of the antennae in the Hippoboscidae it is necessary to compare the structure of their parts with the structure of those of the Muscidae.

The first antennal segment or scape of the Muscidae is ring-shaped; its dorsal surface is slightly broader than the other parts and is provided on the dorso-anterior margin with stiff setae. This segment has neither muscles nor ganglion of its own and is attached to the border of the antennal fossa or socket by a membrane. Through this segment passes the antennal nerve, and to its posterior wall are attached the muscles of the second antennal segment. The second antennal segment or pedicle is conical; its dorsal margin is slightly bi-lobed and there is a furrow which divides the dorsal side longitudinally into the outer and inner halves. The inner half has much longer and thicker setae than the outer. This segment is movable and provided

with a ganglion. It gives off a toothed process (peg of Patton and MacGill, 1925) which serves for the attachment of the third antennal segment or first segment of the flagellum. The flagellum consists of four greatly modified segments. The first is long and covered with sensory setae and bears the olfactory pits. The remaining three segments of the flagellum compose the whip-like structure or arista, which is situated in the dorso-posterior part of the first segment of the flagellum.

All the parts just described for the muscid type of antenna can easily be distinguished in cleared specimens of the antennae of the Hippoboscidae, in which the first antennal segment belongs to the modified scape of the muscid type of antenna. This segment has no muscles or ganglion of its own and is completely fused with the sides of the antennal pit, forming an opening through which the nerve passes into the second antennal segment. In *L. maura* (Pl. XI, figs. 1 and 7) the dorsal part of the first antennal segment is prolonged and covered with long, thin, straw-coloured setae. In *M. ovinus* (Pl. XII, figs. 10 and 12) this segment is much more deeply merged into the head capsule and its dorsal part is overlapped by the posterior border of the antennal pit and is devoid of setae.

The second antennal segment of the Hippoboscidae represents a greatly modified pedicle, the frontal part of which becomes introverted until a deep cavity is formed; the flagellum, following the retraction of its point of attachment, becoming enclosed in this cavity. In *L. maura* this segment is slightly piriform; its dorsal surface has a furrow (*Fu.*) by which it is divided into two unequal parts which correspond to the dorsal halves of the second antennal segment of the muscid type. The inner part is much more developed and forms the so-called dorsal prolongation of the second segment (*D.*). This prolongation is covered with long and darkly pigmented setae, which curve over the anterior border of the antennal pit. Behind these setae there are several straw-coloured short ones. The outer part is less developed; its setae are dark and about twice as short as those in the inner part. Under the dorsal parts there is an opening leading into the cavity where the third antennal segment, attached, as in the Muscidae, to the toothed process, is concealed. The border of the opening is covered with forked spines (Pl. XI, fig. 6) which also spread to the inner wall of the cavity, protecting it from the penetration of foreign particles. The outer surface of this segment is covered with short spines which are very dense in the frontal part. The forked spines represent modified spines of the outer surface, and both remain unconnected with the sensory cells. In *M. ovinus* the second segment is sacciform (Pl. XII, fig. 17); its dorsal surface is devoid of both a dorsal prolongation and a furrow and is covered with setae. The border of the opening of the cavity is protected by forked spines (Pl. XII, fig. 16) which, as in *L. maura*, are modified spines of the outer surface. The internal structure of this segment is the same as in *L. maura* and it has a toothed process (Pl. XII, fig. 20) to which the third antennal segment is attached.

The third antennal segment, or the first segment of the flagellum, is piriform in both *L. maura* and *M. ovinus* and its surface is covered with protecting spines, between which the sensillae are situated. In *L. maura* (Pl. XI, fig. 8) this segment has two tubular olfactory pits (*O.P.*), the shorter one having an opening on the lateral surface, the longer one opening on the dorsal surface. In *M. ovinus* (Pl. XII, fig. 18) there is only one very long and curved olfactory pit (*O.P.*), which opens on the dorsal surface. The arista (*A.*) which, in the antennae of *L. maura*, as well as of *M. ovinus*, consists of a single segment, is, as in the Muscidae, attached to the postero-dorsal part of the third antennal segment and, under normal conditions, protrudes through the opening of the second antennal segment. In *L. maura* it is very narrow in the middle and broadly spatulated at the distal end. In *M. ovinus* the arista is broad at the base, very narrow in the middle, and divided into two branches distally, each branch being further subdivided into comb-like processes. The middle and basal portions of the arista bear a small number of minute spines.

The structure of the antennal groove and the antennae was examined in the following genera of the Hippoboscidae.

PARASITES OF BIRDS	PARASITES OF MAMMALS
Ornithomyia	*Hippobosca*
Ornithoeca	*Ortholfersia*
Ornithophila	*Allobosca*
Olfersia	*Lipoptena*
Lynchia	*Echestypus*
Pseudolfersia	*Melophagus*[1]
Stilbometopa	
Ornithoctona	
Stenopteryx	
Oxypterum	
Myophthiria	
Brachypteromyia	

On the basis of the study of the species belonging to the above-mentioned genera the antennal pit may be divided into two types—the open antennal pit and the closed antennal pit.

The open antennal pit is represented by *L. maura* in this work. It is characterised by the absence of a sharp margin in its anterior part. Those Hippoboscidae which are parasites of birds have the open antennal pit. Moreover, in *Ornithoeca*, *Ornithophila*, *Olfersia*, *Pseudolfersia*, *Lynchia*, *Stilbometopa*, *Stenopteryx*, *Oxypterum*, *Myophthiria* and *Brachypteromyia* the dorsal part of the first antennal segment has a small prolongation which is covered

[1] It must be noted here that, according to Froggatt (1909), "The wallaby fly (*Olfersia macleayi* Leach)" infests wallabies in New South Wales, but according to Major E. E. Austen (verbal communication) Froggatt's species is really *Ornithomyia avicularia* L. Moreover, *Hippobosca struthionis* Janson appears to be a parasite of ostriches in Cape Colony (Ormerod, 1889).

with setae. In *Ornithomyia* and *Ornithoctona* the first antennal segment is indistinct. Massonnat (1909) in his work has shown that the parasites of birds have a dorsal prolongation in the second antennal segment. This prolongation has been described in connection with the description of the second antennal segment of *L. maura*. The same structure is present in the following genera: *Ornithomyia, Ornithoeca, Ornithophila, Olfersia* and *Pseudolfersia*. In *Stenopteryx, Oxypterum, Myophthiria* and *Brachypteromyia* the prolongation of the dorsal part of the second antennal segment is much more developed. It is broadened distally and has a rounded anterior border, its ventral surface is slightly concave and the dorsal surface is covered with long setae which are more dense in the anterior part. This prolongation overlaps the antennal pit dorsally and projects in front of its anterior margin. In *Stilbometopa* and *Ornithoctona* the prolongation of the second antennal segment is strongly chitinised and has an irregularly triangular shape.

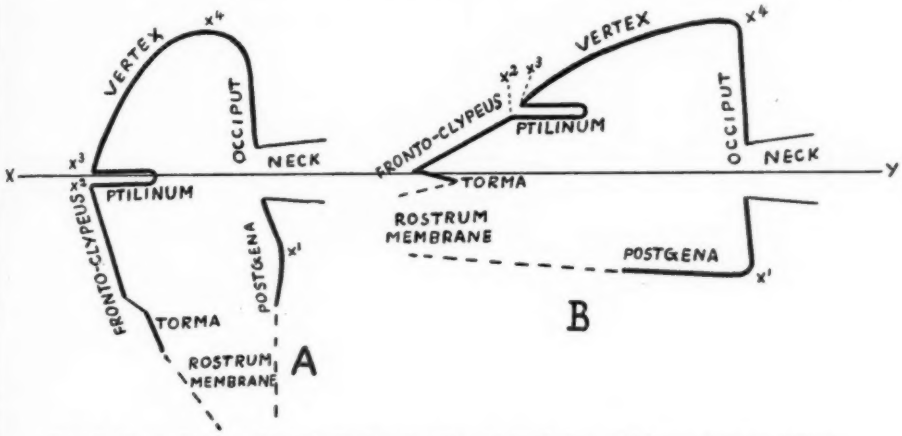
The closed antennal pit is represented in *M. ovinus*. This type of pit has a very distinct border in its anterior part and is completely separated from the gena by the ptilinal suture. This pit has been found in the following parasites of mammals: *Hippobosca, Lipoptena, Echestypus* and *Melophagus*. In the closed antennal pit the dorsal part of the first antennal segment is partly hidden under the posterior border of the pit, and only in *Hippobosca* has the second segment a scarcely perceptible furrow in the dorsal part. In *Lipoptena, Echestypus* and *Melophagus* the second antennal segment is completely merged into the antennal pit.

Two genera parasitic on mammals, *Ortholfersia* and *Allobosca*, have the open antennal pit. The first segment of the antennae of *Ortholfersia* is indistinct, while in *Allobosca* it has a marked prolongation dorsally. The dorsal prolongation of the second antennal segment of *Ortholfersia* is larger than in *L. maura*, whereas in *Allobosca* this prolongation is strongly developed and is broad and flat; its anterior border is sharply curved and bears thick setae.

The study of the antennal structure in the Hippoboscidae shows that they have a greatly modified muscid type of antenna. These modifications have arisen in consequence of the adaptation of these flies to a parasitic mode of life and serve to protect the most important parts of the antenna, such as the flagellum, when these flies move amongst the feathers or hairs of the host. This protection was ensured by the formation of the antennal pit and the retraction of the flagellum into the pedicle. Moreover, in those flies which have the open antennal pit there is a prolongation of the dorsal portion of the pedicle. This prolongation overlaps the anterior part of the antennal pit and apparently serves to shield the arista from the feathers or hairs which the fly encounters during progression. When the dorsal prolongation of the second antennal segment is not very long, as in *L. maura*, then its anterior part bears many long, curved setae, which also serve for the protection of the arista. In the other pupiparous family, Nycteribiidae, the type of antenna is identical with that in the Hippoboscidae.

ON THE MODIFICATION OF THE HEAD CAPSULE.

It has already been shown that the head capsule and the antennae of the Hippoboscidae have very much in common with the same parts of the Muscidae, and the head capsule of the Hippoboscidae must be regarded as the head capsule of the Muscidae greatly modified in connection with the adaptation to a parasitic mode of life. The modification of the shape of the head was doubtless correlated with the modification of the entire body from a more or less cylindrical to a dorso-ventrally compressed form. This form of body is a common condition in all parasitic insects except the Siphonaptera, in which the body is laterally compressed. The dorso-ventral flattening was developed by degrees as the insect acquired a firmer mode of attachment to its host. It is not necessary for the Muscidae to be able to attach themselves firmly, and



Text-fig. 1. *A*, diagram of longitudinal section through head of muscid type; *B*, same of hippoboscid type; *XY*, longitudinal body axis.

therefore the shape of their body and head is quite different from those of the Hippoboscidae.

Text-figure I shows diagrammatic longitudinal median sections through (*A*) the head of the muscid type and (*B*) the head of the hippoboscid type. Both heads are arranged in such a way that the longitudinal body axis (*XY*) of these flies lies in the same plane. This axis passes through the middle of the occipital foramen in both heads and their occipita are more or less perpendicular to the axis; on the other hand, the other regions of the head are differently arranged in these two types. The head capsule of the muscid type extends dorso-ventrally, the mouth parts being directed downwards (*A*); this type of head is called hypognathous (Macgillivray, 1923). The head of the hippoboscid type is, on the other hand, dorso-ventrally compressed and lies in a horizontal position with the mouth parts directed forwards (*B*); this type of head is

called prognathous (Macgillivray, 1923) and represents a modification of the hypognathous type.

In the muscid type the dorsal surface of the head is dome-shaped, whereas in the hippoboscid type it is considerably flattened and the vertex forms a right angle (X^4) with the occiput. Moreover, in the hippoboscid type the angle X^3 is smaller and the angle X^2 larger in the region of the ptilinum than in the muscid type, in consequence of which the fronto-clypeus and the vertex form an almost continuous flat surface which gradually slopes down to the axis. The lateral and the ventral surfaces of the head have undergone changes parallel with the dorsal surface. In the muscid type, the postgenae have a bend (*A*, X^1) in the middle, below the occipital foramen. In the hippoboscid type the anterior part of the postgenae has been raised, forming a right angle (*B*, X^1) with its posterior part. The posterior part of the postgenae occupies the same position with regard to the axis in both types of head. The posterior surface of the head of the hippoboscid type is triangular; this shape was produced by the lateral broadening of the vertex and by the contraction of the postero-lateral parts of the postgenae at the points where they form depressions with smooth surfaces for the greatly developed coxae of the anterior pair of legs. Owing to this peculiarity the head of the Hippoboscidae appears to be withdrawn into the thorax. These depressions of the postgenae are specially developed in the following genera of the Hippoboscidae: *Lipoptena*, *Echestypus* and *Melophagus*. A greatly flattened head has been found in the genus *Allobosca*, in which the body is more flattened than in all the other genera which have been examined.

The modification of the anterior parts of the postgena has been mentioned in the description of the head of *Lynchia maura* and *Melophagus ovinus*. The modification of the fronto-clypeus and the torma (Peterson, 1916) will be described together with the mouth parts.

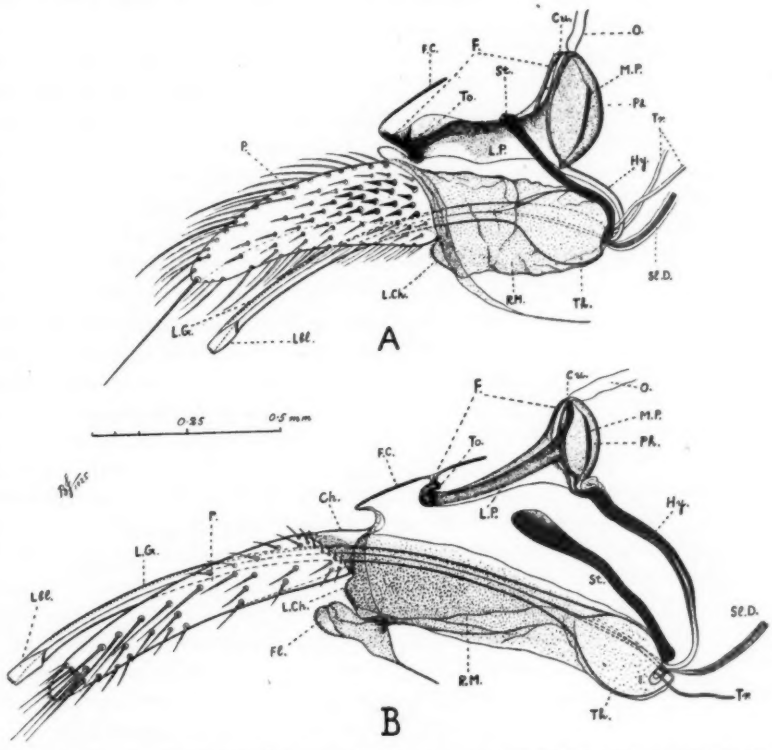
The Mouth Parts. General Account.

(Text-fig. II, *A* and *B*.)

The proboscis of the Hippoboscidae is composed of the same parts as the proboscis of the Muscidae, the structure of these parts showing a specially close resemblance to those of the blood-sucking Muscidae. A detailed study of the proboscis can only be made by dissecting it out, because its proximal portion lies in the head capsule, whilst the protruding distal parts are concealed between the maxillary palps which alone can be seen in total preparations. The homologies of the chitinous parts and the muscles will be discussed later in connection with their detailed description.

The proboscis is divided into three regions, viz. the *rostrum*, the *haustellum* and the *labellum*. The rostrum (basiproboscis of Peterson, 1916) is composed of the following parts: the rostrum membrane (*R.M.*), the maxillary palps (*P*), the fulcrum (*F.*), the hyoid (*Hy.*), the stipites (*St.*), and the anterior portion of the salivary duct with the salivary valve and the muscles. All these parts

except the maxillary palps lie in the head capsule. In *Melophagus ovinus*, in consequence of the elongation of the haustellum, the proboscis membrane, the stipites and the hyoid are considerably longer than the corresponding parts in *Lynchia maura*. The *haustellum* (medioproboscis of Peterson, 1916) includes the labrum-epipharynx, the hypopharynx and the labium. The latter is composed of two parts—the ventral or the theca (*Th.*) and the dorsal or the



Text-fig. II. *A*, proboscis of *Lynchia maura* Bigot; *B*, same of *Melophagus ovinus* L. Both from cleared preparations.

labial gutter (*L.G.*), which was described and named by Stephens and Newstead (1907) in *Stomoxys*. The labrum-epipharynx and the hypopharynx are concealed in the labial gutter, on account of which the study of these parts can only be made by dissecting them out. The *labellum* (*Lbl.*) (distiproboscis of Peterson, 1916) is the distal part of the proboscis and is attached by a membrane to the anterior end of the haustellum. It is devoid of pseudotracheae and has prestomal teeth.

The Rostrum.

(Text-fig. II; Pls. XI, XII and XIII.)

The *rostrum membrane* (*R.M.*) may be divided into an outer and an inner part. The outer part of the rostrum membrane of the Hippoboscidae corresponds to that part of the rostrum membrane of the Muscidae which is attached to the border of the proboscis aperture and is limited in front by the palps and on the sides by the narrow stripes resulting from secondary chitinisation (St.-2 of Peterson, 1916; palpifer of Frey, 1921). In the Hippoboscidae this part of the rostrum membrane occupies a very small space outside the head capsule. In *Lynchia maura* the attachment of this part of the rostrum membrane on the ventral side of the head is similar to that of *Melophagus ovinus*. It is continuous with the median membranous part of the head capsule and extends from the membranous flap (*Fl.*) to the lateral chitinised parts (*L.Ch.*). The dorsal and the dorso-lateral parts of this membrane in *Lynchia maura* are attached to the genae and to the torma (*To.*), from which the membrane extends to the maxillary palps. In *Melophagus ovinus*, in consequence of the displacement of the torma and the reduction of the genae, this membrane is attached to the fronto-clypeus and those parts of it which lie posteriorly to the maxillary palps are slightly chitinised (*Ch.*). The inner part of the rostrum membrane of the Hippoboscidae corresponds to the anterior part of the same membrane in the Muscidae, which is limited posteriorly by the palps and the lateral chitinised parts and is attached anteriorly to the haustellum. In the Hippoboscidae this part of the rostrum membrane is completely inverted into the head capsule and encloses the posterior part of the haustellum when the proboscis is at rest. In *Lynchia maura* this part of the rostrum membrane is cone-shaped, its surface being covered with small scales broadened at the base, which are more numerous in the lateral slightly chitinised parts. The chitinisation of the latter is very marked ventrally to the base of the maxillary palps, gradually disappearing towards the haustellum. In *Melophagus ovinus*, on account of the elongation of the haustellum, this part of the rostrum membrane is much more developed than in *Lynchia maura*. In transverse sections it is shaped like an inverted T (Pl. XV, fig. 54) and its dorsal and ventral parts are very flexible, whereas the lateral chitinised parts, which correspond with those of *Lynchia maura* and are also covered with scales, are more developed than in the latter. From the position of these lateral chitinised parts it may be assumed that they are homologous with Peterson's St.-2 (1916) and the chitinised parts of the rostrum membrane which, in the Muscidae, lie ventrally to the maxillary palps. The anterior part of the rostrum membrane is attached to the proximal border of the labrum and the theca. When the proboscis in *Lynchia maura* is protracted, the rostrum membrane is completely everted, exposing the bulbous part of the haustellum. In *Melophagus ovinus* the rostrum membrane is not everted completely and the bulb remains enclosed by the sac formed from the incompletely everted rostrum

membrane, under the posterior parts of the maxillary palps (Text-fig. IV, *B*).

The *maxillary palps* (*P.*) of the Hippoboscidae, as in all the other Cyclorrapha, consist each of a single joint. They are attached to the dorsal surface of the rostrum membrane at the posterior border of the lateral chitinised parts and are directed forwards. In the resting position they are applied to each other as in tsetse-flies (*Glossina*), their membranous and concave inner sides forming a cavity in which the distal part of the haustellum and the labellum lie when the proboscis is at rest. Their size and form vary in different genera of the Hippoboscidae: in many forms they are elongated and distinctly visible, while in *Allobosca crassipes* Speiser they are very small and almost concealed under the anterior part of the fronto-clypeus. The outer surface of the palps is convex, strongly chitinised and covered with several setae. In *Lynchia maura* (Text-fig. II, *A* and Pl. XIV, fig. 39) the palps are slightly curved and broadened at the base and their dorsal and ventral parts have long setae, while the lateral parts are covered with short setae; also there are a number of stout and very dark setae in the proximal part of the lateral surface and a very long terminal seta at the tip. All the setae of the outer surface possess a distinct theca (*T.S.*). At the inner side of each theca there is a large sensory cell connected with a nerve. The membranous inner surface of the palps is covered with very fine bristles and has a T-shaped membranous process running along the middle. These processes serve to hold the distal part of the haustellum in the desired position. The maxillary palps of *Melophagus ovinus* (Text-fig. II, *B*; Pl. XV, fig. 53) are somewhat finger-shaped and much longer and narrower at the base than in *Lynchia maura*. Their outer surface is covered with strong setae, which are more numerous and longer at the distal end. The tips of the palps are membranous and are covered with very minute bristles. The maxillary palps have no muscles of their own and they are brought into motion by the movements of the rostrum and the haustellum.

The *stipites* (*St.*), or apodemes of many authors, are very similar in structure to those of the Muscidae. Peterson (1916) says of the Calyptratae: "The free so-called apodeme is unquestionably derived from the ental ingrowth of the stipes." In the Muscidae the proximal end of the stipes is fused with the rudimentary galea, which is movably attached to the labrum. In the Hippoboscidae the galea is indistinct and apparently fused with the proximal end of the stipes. The stipes of *Lynchia maura* (Pl. XIII, fig. 21) is a densely pigmented curved rod, both ends of which are spatulated; it is movably attached to the lateral process of the labrum and to the dorsal part of the rostrum membrane in its anterior part. In *Melophagus ovinus* (Pl. XIII, fig. 27) the stipes is one-fourth longer than in *Lynchia maura* and its distal end is broadly spatulated, whereas the proximal end is round and attached to the socket of the lateral process of the labrum. It is connected with the rostrum membrane as in *Lynchia maura*.

The *fulcrum* (*F.*) in the Hippoboscidae is well developed and is composed

of (*a*) the torma (*To.*) (Peterson, 1916) or anterior arch (Cragg, 1912, 1913), (*b*) two lateral plates (*L.P.*) or paratormae (Macgillivray, 1923) fused together and connecting the torma with the (*c*) pharynx (*Ph.*), which itself is composed of an anterior and a posterior plate. In many genera of the Hippoboscidae the fulcrum is of the same type as in the Muscidae, but it has a different connection with the rostrum membrane and the configuration of the parts forming the fulcrum also differs in details. The torma forms the anterior part of the fulcrum and in *Lynchia maura* and *Hippobosca equina* it is distinctly arched. The sides of the arch are parallel, its dorsal parts being thick and round, while the ventral are membranous. The anterior part of the torma is shown in Pl. XIII, fig. 23, to have anterior and posterior projections, its ventral surface being flat. The dorsal part of the anterior projection is attached by a hyaline filament to the anterior border of the fronto-clypeus. The rostrum membrane is not connected with the lateral parts of the arch as in the Muscidae, but is attached around the anterior part of the torma. The lateral plates (*L.P.*) have the same structure as the lateral parts of the torma with which they are fused. The posterior parts of the lateral plates are broader than the anterior parts and are fused with the posterior plate or wall of the pharynx (*Ph.*) along its lateral borders (Pl. XIII, fig. 25). The posterior plate is slightly oblong and concave and is pigmented and rigid; its lateral borders are curved inwards, forming in the posterior part two cornua (*Cu.*), which, in the Hippoboscidae, are less developed than in the Muscidae. Between the cornua the posterior plate is arched. The anterior plate represents the invaginated anterior wall of the pharynx and it is continuous with the posterior plate along its lateral borders, which are really the folds produced by the invagination. This plate is hyaline and flexible and it is much thicker in the middle around the median plate (*M.P.*). The median plate is pigmented, somewhat rhomboid in form and has a very rough margin. It is inserted in the middle of the anterior plate.

The anterior part of the oesophagus (*O.*) is attached between the cornua to the anterior plate and to the arched border of the posterior plate of the pharynx, where it is surrounded by the fibres of the *sphincter of the oesophagus* (*S.O.*). Before passing through the cephalic ganglion the oesophagus first bends forwards and upwards and then downwards.

The fibres of the *dilators of the pharynx* (*D.Ph.*) or the pumping muscles occupy the space between the lateral plates of the fulcrum, being attached to the inner sides of the lateral parts of the torma and their short and hyaline tendons inserted into the median plate. When these muscles contract they pull the anterior plate from the posterior, producing between them a cavity. After the relaxation of these muscles the anterior plate becomes depressed and is in contact with the posterior plate.

The fulcrum of *Melophagus ovinus* is modified (Text-fig. II, *B*; Pl. XIII, figs. 28 and 30). The torma is attached to the middle of the posterior part of the fronto-clypeus and there is no connection with the rostrum membrane at all. The displacement of the torma is the consequence of the modification of

the fronto-clypeus. Apparently the ancestors of *Melophagus ovinus* had an incurved fronto-clypeus, as in *Hippobosca*, *Lynchia*, *Olfersia* and *Ornithophila*. Gradually the sides of the incurvature or bay were brought nearer and formed the fissure, as in *Stilbometopa* and *Pseudolfersia*. In *Melophagus ovinus* the sides of the fissure are fused together, the line of union being represented by the faint median furrow mentioned on page 324. This furrow has the same form as the fissure of the fronto-clypeus of *Stilbometopa* and *Pseudolfersia*. In *Lypoptena* and *Echestypus* the torma is attached, as in *Melophagus*, to the posterior end of the furrow on the inner side of the fronto-clypeus. In *Allobosca* the torma is also displaced but the furrow is indistinct. In connection with the displacement the torma of *Melophagus ovinus* has become markedly modified, its anterior part being bent backwards and more or less firmly attached to the fronto-clypeus. The lateral parts do not form a distinct arch as in *Lynchia* and *Hippobosca* but spread widely and make nearly a right angle with the lateral plates of the fulcrum. The lateral plates are much narrower anteriorly and form very broad lateral projections at the borders of the posterior plate of the pharynx, which is concave and nearly circular. The anterior and the median plates of the pharynx have the same structure as in *Lynchia maura*. The *sphincter of the oesophagus* and the *dilators of the pharynx* are well developed.

The *hyoid* (*Hy.*). In the Orthorrhapha the pharynx is connected with the epipharynx and the hypopharynx, whereas in the Cyclorrhapha the pharynx is separated from these by the secondary sclerite which has been named the hyoid by Lowne (1890–92) in *Calliphora*, and the buccal cavity by Cragg in the blood-sucking Muscidae. The origin of the hyoid is not quite clear. According to Peterson (1916) it has originated from either the epipharynx or the hypopharynx. In *Lyperosia*, *Stomoxys* and *Haematobia* the hyoid is represented by a trachea-like tube. In *Musca*, *Calliphora*, *Sarcophaga* and *Scatophaga* the hyoid is a small sclerite, the ventral wall of which is densely pigmented and thicker than the membranous dorsal wall.

In the Hippoboscidae this secondary sclerite is much better developed than in any other Cyclorrhapha and its development is undoubtedly closely connected with the development of the retractibility of the proboscis. In *Lynchia maura* (Pl. XIII, fig. 26; Pl. XIV, fig. 41) the hyoid is a tubular sclerite, the diameter of its lumen being 21·5 μ in the anterior part and 25·2 μ in the posterior part; its ventral wall (which occupies a more or less dorsal position when the proboscis is retracted) is thick and chitinised. This chitinisation, however, gradually disappears in the anterior part of this wall, which is membranous like the dorsal wall. The posterior end of the hyoid is movably attached to the pharynx, whereas the anterior part is connected with the epipharynx and the hypopharynx, as in the Muscidae, and the lumen is continuous with the food canal of the haustellum.

In *Melophagus ovinus* the hyoid has the same structure as in *Hippobosca equina* and *Lipoptena* but is much longer, it being four times the length of that in *Lynchia maura*. Its ventral wall is thin and chitinised and its dorsal wall

is thicker than the ventral, while the lateral walls are extremely thick and appear in the form of two flattened bars (Pl. XIII, fig. 28; Pl. XV, fig. 55). The broad posterior end of the hyoid has a distinct joint with the pharynx, its anterior part is membranous and much narrower than the posterior, and its lumen has the same dimensions as in *Lynchia maura*.

The *salivary duct* (*Sl.D.*) and the *salivary valve* (*Sl.V.*). The part of the duct which runs from the salivary glands to the salivary valve is composed of a chitinous intima (*C.I.*) and a single layer of hypodermic cells. The intima is provided with spiral taenidia (*Ta.*) in *Lynchia maura* but in *Melophagus ovinus* taenidia are absent in this part of the duct. Between the salivary valve and the hypopharynx the duct is broad: its lumen is 10μ in diameter in *Lynchia maura* and 7·5μ in *Melophagus ovinus*. The intima is thick, the taenidia are markedly developed and the hypodermic layer consists of large cells. This part of the duct is closely applied to the ventral wall of the hyoid when the proboscis is protracted. Before entering the hypopharynx the duct becomes narrower and the taenidia disappear. The salivary valve (Pl. XIV, fig. 38) is formed by the invagination of the wall of the duct, and its structure is the same in both *Lynchia maura* and *Melophagus ovinus*.

Two *salivary valve muscles* (*Sl.V.M.*) [dilatores ductus salivalis (Müggenburg, 1892)] are inserted in the hollow formed by the invagination of the dorsal wall of the duct, and each of these muscles is attached to the lateral projections of the lateral plate. When these muscles are relaxed the dorsal part of the valve is in touch with the ventral part and the duct is closed, but when they contract they pull the dorsal part from the ventral, thus opening the duct.

The Muscles of the Rostrum.

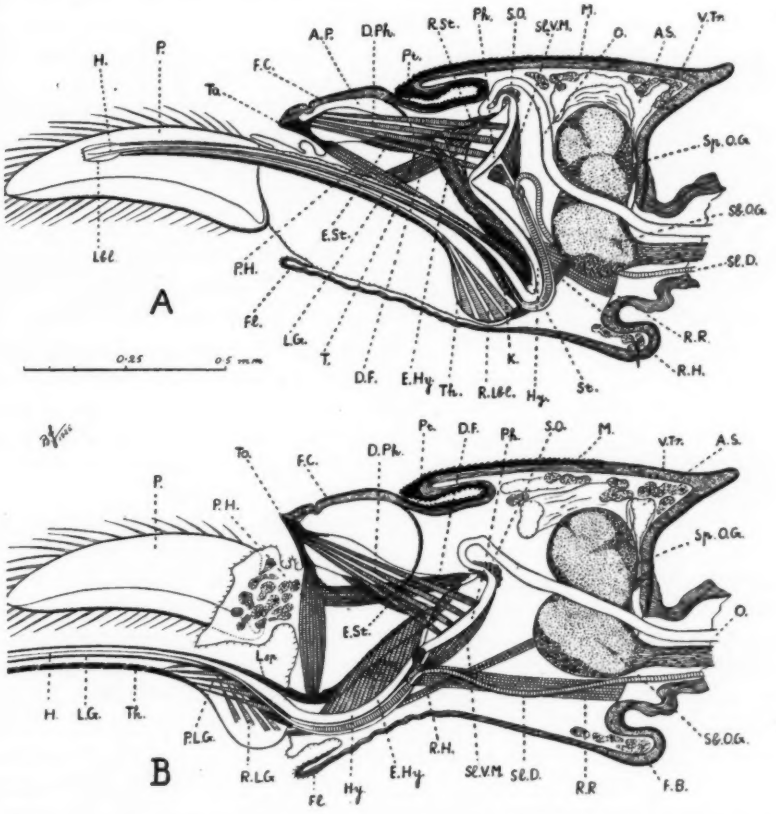
(Text-figs. III, *A* and *B*, and IV, *A* and *B*.)

The chitinous parts of the rostrum are movably attached to each other and represent a system of levers which are movably connected by the anterior part of the fulcrum (torma) with the fronto-clypeus, and by the anterior membranous part of the hyoid with the haustellum, which in its turn is connected with the rostrum membrane. This system can be brought into action by sets of muscles which, according to Kraepelin (1883), act as a parallelogram of forces. In the Hippoboscidae these sets of muscles are the same as in the Muscidae. However, in the former the action of some muscles is quite different from those in the Muscidae in consequence of the modification of the form of the head capsule and the position of the chitinous parts of the rostrum. Moreover, the protraction of the proboscis depends entirely upon the action of the muscles, and not upon the respiratory system as in the Muscidae. This has also been pointed out by Massonnat (1909).

The following muscles have been found in *Lynchia maura* and *Melophagus ovinus*:

The *protractors of the haustellum* (*P.H.*) [protrusores proboscidis (Müggenburg, 1892); flexors of the labrum-epipharynx in *Musca* (Hewitt, 1914)].

These muscles arise from the anterior border of the middle part of the torma and are inserted into the anterior part of the stipites at the point of attachment of the rostrum membrane in *Lynchia maura*. In *Melophagus ovinus* they are inserted, as in the Muscidae, into the lateral processes of the labrum-epipharynx, where they are connected with the stipites.



Text-fig. III. Right half of head of *Lynchia maura* Bigot, showing muscles of rostrum and haustellum; *A*, proboscis retracted, protractor and retractor of labial gutter not shown; *B*, proboscis protracted, retractor of labellum not shown.

The *depressors of the fulcrum* (*D.F.*) [depressores fulcri (Müggenburg, 1892)]. These muscles were described in *Musca* as the retractors of the fulcrum (Hewitt, 1914) and under the same name in the lower Schizophora (Frey, 1921), though in *Glossina* they were described as being depressors of the fulcrum (Hansen, 1903). In Hippoboscidae these muscles rotate the fulcrum in the opposite direction to those of the Muscidae, in consequence of the modification of the head capsule, which changed the position of the torma relative to

the points of attachment of these muscles. In *Lynchia maura* each muscle arises from the anterior end of the gena, whereas in *Melophagus ovinus* they arise from the anterior border of the fronto-clypeus. Each of these muscles passes along the inner side of the antennal pit and is inserted into the posterior border of the lateral projection of the lateral plates.

The *elevators of the stipites* (*E.St.*) [levatores maxillarum (Müggenburg, 1892); accessory flexors of the haustellum in *Musca* (Hewitt, 1914)]. Each of these muscles arises from the lateral part of the torma and is inserted into the distal end of the stipes.

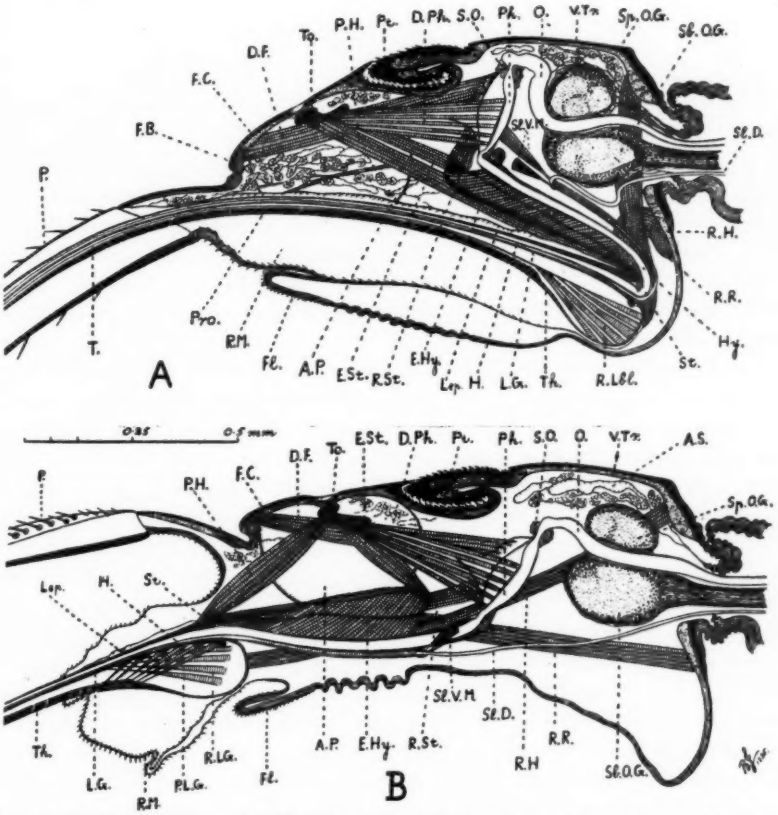
The *retractors of the stipites* (*R.St.*) [retractores maxillarum (Müggenburg, 1892)] correspond to the extensors of the haustellum in *Musca* (Hewitt, 1914). These muscles arise from the lateral borders of the posterior plate of the fulcrum, and each is inserted into the distal end of the stipes.

The *elevators of the hyoid* (*E.Hy.*) [protrusores maxillarum (Müggenburg, 1892)]. These muscles arise from the lateral walls of the hyoid, and are inserted into the posterior third of the stipites. They are very strong in *Lynchia maura* and in *Melophagus ovinus*. These muscles have not been described in the Muscidae and are apparently homologous with the extensors of the haustellum which in *Musca* and *Calliphora* are very strong, each of them being represented by two very distinct bands. It is probable that in the Hippoboscidae the position of one of the bands was changed and instead of being inserted into the lateral border of the anterior plate it became attached to the hyoid.

All the muscles just described serve to protract the haustellum. They do not all contract simultaneously. The retractors of the haustellum contract first and in so doing pull the haustellum through the inverted rostrum membrane and thereby draw out the hyoid, which becomes continuous with the haustellum, occupying a more or less horizontal position. The elevators of the hyoid and the elevators of the stipites act together in the unbending and lifting of the middle part of the hyoid. The contractions of the depressors of the fulcrum lower it and, as the hyoid connects the fulcrum with the haustellum, the latter is pushed out still further by the action of the depressors. The elevators and the retractors of the stipites also act together with the protractors of the haustellum in the rotation of the latter. During the protraction of the proboscis the membrane of the ventral part of the head becomes invaginated into the head capsule. This invagination is much greater in *Melophagus ovinus* than in *Lynchia maura*. Moreover, there is another interesting change in the dorsal part of the head of *Melophagus ovinus*. When the proboscis is protracted the convexity in the region of the ptilinum disappears and the dorsal surface of the head becomes quite flat. This phenomenon depends on the actions of the muscles of the rostrum and the flexibility of the membranous parts of the head.

The *retractors of the rostrum* (*R.R.*) [retractores proboscidis inferiores (Müggenburg, 1892); retractors of the rostrum in *Musca* (Hewitt, 1914);

retractores rostri in lower Schizophora (Frey, 1921)]. These muscles are very strong and when relaxed are nearly four times longer than when contracted. In *Lynchia maura* and in *Melophagus ovinus* they arise from the thickenings on the sides of the occipital foramen and are inserted into the proximal part of each stipes.



Text-fig. IV. Right half of head of *Melophagus ovinus* L., showing muscles of rostrum and haustellum; *A*, proboscis retracted, protractor and retractor of labial gutter not shown; *B*, proboscis protracted, retractor of labellum not shown.

The *retractors of the haustellum* (*R.H.*) [retractores proboscidis superiores (Müggenburg, 1892); retractors of the haustellum in *Musca* (Hewitt, 1914)]. In *Lynchia maura* and in *Melophagus ovinus* these muscles have the same attachments as in the Muscidae, arising from the thick dorso-lateral part of the occiput and being inserted into the dorso-posterior margin of the theca. In the relaxed condition these muscles are the longest. In *Lynchia maura* they are less developed than in *Melophagus ovinus*. In contracting, these

two pairs of muscles withdraw the haustellum into the head and bend the hyoid which, in its turn, pushes the fulcrum upwards. The retractors of the haustellum also serve to turn the haustellum towards the sides. It must be noted that in *Melophagus ovinus* the torma is almost immovably attached to the fronto-clypeus and therefore its lateral parts act as a spring during the elevation of the fulcrum.

The Haustellum.

(Text-fig. II, *A* and *B*; Pls. XIII, XIV and XV.)

The structure of the haustellum in the Hippoboscidae closely resembles that of *Glossina*. The parts which compose this region of the proboscis are very long, narrow and strongly chitinised and they represent true mouth parts which have undergone modification to enable them to penetrate easily into the skin of the hosts. In total preparations the haustellum is seen as a slightly curved rod, the proximal part of which is bulbous and the rest cylindrical, the shortest diameter being 32μ in *Lynchia maura* and 25μ in *Melophagus ovinus*. The length of the haustellum varies in different genera of the Hippoboscidae: in *Hippobosca equina* and *Lynchia maura* its length with the labellum is 1·2 mm., in *Melophagus ovinus* 1·8 mm. and in *Lipoptena* 0·9 mm. On each side of the haustellum there is a distinct furrow stretching along its narrow part from the bulb to the distal end. This furrow is formed by the junction of the labial gutter (*L.G.*) and the theca (*Th.*). The latter two parts of the haustellum represent greatly modified parts of the labium, and some stages of their modification can be traced by comparing the structure of these parts in the blood-sucking Muscidae. According to Crampton (1925), the labium of the Diptera is composed of the labial palps, palpigers and mentum. This author regards the labellum as a modification of the labial palps. Thus the theca of the Diptera is formed by the palpigers and mentum. The theca of *Lynchia maura* and *Melophagus ovinus* has a guttered appearance in transverse sections (Pl. XIV, figs. 35 and 36; Pl. XV, figs. 47 and 48) and is very thick and rigid. Towards the bulb the theca becomes slightly broader and thicker on its ventral side. The bulbous part of the theca is very thin and is globular in *Hippobosca equina* and *Lynchia maura* (Pl. XIII, fig. 22) and slightly ovoid in *Lipoptena* and *Melophagus ovinus* (Pl. XIII, fig. 29). The lateral parts of the bulb are bent dorsally over the labial gutter and the labrum-epipharynx, its borders on the dorsal surface being bent inwards and connected by a flexible membrane with the lateral parts of the labial gutter. The posterior part of the labial gutter is connected by thick fibrous tissue with the posterior part of the border of the theca, forming two openings through which the tracheae and the labial nerves pass into the bulb. In *Lynchia maura* the part of the labial gutter which lies in the bulb has a very distinct keel (*K.*) and two lateral processes to which are attached the muscles of the haustellum (Pl. XIV, figs. 42 and 43). In *Melophagus ovinus* the keel is absent and the lateral processes are very small. In the narrow part of the haustellum the labial gutter lies above the theca, its

toothed lateral parts being bent over the dorsal surface of the labrum-epipharynx. These lateral parts gradually approach each other until the borders become adjacent towards the anterior end. The labial gutter passes through the labellum and its dorso-lateral parts are connected with the latter by a flexible membrane (Pl. XIV, fig. 37; Pl. XV, figs. 50 and 51). This membrane also connects the proximal part of the labellum with the anterior end of the theca, forming between them a very distinct joint. From this joint the membrane passes along the sides of the haustellum into the bulbous part, connecting the dorsal borders of the theca with the ventral parts of the labial gutter. Thus the theca, the labial gutter and the labellum are movably connected with each other.

Since the haustellum is adapted for penetration into the skin, the surfaces of the labial gutter and the theca in their narrow parts are smooth and devoid of setae. Although two rows of very minute spines are present on the ventral surface of the theca, each spine is situated at the bottom of a small pit and is connected through a pore (*Pro.*) in the chitin with a sensory cell which in its turn is connected with the labial nerve. I have compared these pores with those of *Glossina* and could find no difference in their structure. The second type of spine described by Stephens and Newstead (1906) in *Glossina* is evidently due to a misinterpretation of the parts seen.

In *Lynchia maura* there are two small setae on the ventral surface of the anterior part of the bulb and very minute bristles arranged in groups on the membrane of the bulb between the dorsal border of the theca and the labial gutter (Pl. XIII, fig. 24). These bristles belong to the "organe mûriforme" described by Massonnat (1909). According to this author it is present in *Hippobosca*, *Lynchia*, *Ornithomyia* and *Cratarrhina* (*Oxypterum* Leach) and absent in *Lipoptena* and *Melophagus*. This structure is composed of a mass of elongated cells which undoubtedly represent modified hypodermic cells. Each cell has a comparatively large nucleus situated in its broader part. The protoplasm of the narrow part of the cell exhibits a fibrillar structure and is connected through the membrane with the bristles (Pl. XIV, figs. 40, 42 and 43). I could find no connection with the nerve and the function of this organ remains unknown.

The *labrum-epipharynx* (*Lep.*) is stylet-shaped and its proximal part is strongly chitinised and rigid, whereas the distal end is membranous and very flexible. The labrum-epipharynx is formed by the fusion of the labrum (*L.*) and the epipharynx (*Ep.*). At the base the labrum is firmly attached to the labial gutter, while its thick proximal part is arched and has two lateral processes which are attached to the stipites (Pl. XIII, figs. 21 and 27). The epipharynx, arising from the membranous anterior part of the hyoid, forms an almost closed tube and its membranous borders become fused with the ventral membranous borders of the lateral part of the labrum. A little further on the dorsal part of the epipharynx fuses with the dorsal part of the labrum and two cavities are formed between their walls (Pl. XIV, fig. 40; Pl. XV,

fig. 56). There are no fan-shaped muscles or dilators of the labrum-epipharynx as in *Musca*. The cavities are occupied by hypodermic cells, nerves and tracheae.

In the bulbous part of the haustellum the surface of the epipharynx bears very minute bristles projecting into the food canal as in *Glossina* (Stephens and Newstead, 1906, described them as hair-bearing papillae situated on the dorsal side of the labrum). These bristles are connected through pores in the wall of the epipharynx with sensory cells which are situated in the cavity of the labrum-epipharynx.

In *Lynchia maura* the bristles are not numerous and are situated on the sides, while in *Melophagus ovinus* they are scattered irregularly and are more numerous. Towards the anterior end of the labrum-epipharynx its cavities gradually disappear, the labrum and the epipharynx completely fusing and no suture being left between them in the distal part.

The position of the labrum-epipharynx is best seen in transverse sections of the haustellum. It lies in the labial gutter and the teeth which are formed by the labrum on its lateral surfaces are interlocked with the teeth of the lateral walls of the labial gutter, as in *Glossina*. At the distal end of the labrum-epipharynx these teeth appear in small number on the dorso-lateral surfaces, but proximally their number increases and they become displaced to the sides. In *Lynchia maura* the labrum-epipharynx is longer than the theca and therefore its distal end lies in that part of the labial gutter which is in the labellum, while in *Melophagus ovinus* the labrum-epipharynx is slightly shorter than the theca.

The *hypopharynx* (*H.*) is nearly as long as the combined haustellum and labellum in *Lynchia maura* and in *Melophagus ovinus*. It is a very slender and hyaline mouth part, the upper end of which is completely fused with the basal part of the labial gutter (Pl. XIV, fig. 40). The suture on the line of this fusion makes its appearance when the hypopharynx becomes distinct as a separate organ overlying the gutter (Pl. XIV, fig. 42; Pl. XV, fig. 56). The hypopharynx is perforated by the salivary duct (*Sl.D.*). The lumen of this part of the duct has a diameter of 3·0μ in *Lynchia maura* and 2·0μ in *Melophagus ovinus*. The duct opens on the dorsal side of the hypopharynx at its narrow distal end which lies in the labial gutter not far from its anterior border. It can be seen from transverse sections of the haustellum that the hypopharynx lies in the shallow groove of the labial gutter (Pl. XIV, figs. 35, 36 and 43; Pl. XV, figs. 47 and 48). When the hypopharynx in *Lynchia maura* becomes separated from the labial gutter its flat lateral parts become inserted in the grooves formed by the ventral borders of the labrum-epipharynx which have originated by the fusion of the labrum with the epipharynx (Pl. XIV, figs. 36 and 43). In the distal part of the haustellum these grooves disappear and the borders of the labrum-epipharynx lie in contact with the dorsal surface of the hypopharynx. In *Melophagus ovinus* the grooves are absent and the ventral borders of the labrum-epipharynx lie in contact with the dorsal surface of the

hypopharynx throughout their entire length (Pl. XV, figs. 47 and 48). The arrangement of these two mouth parts shows that they form the food canal in the haustellum.

THE LABELLUM AND THE PRESTOMAL TEETH.

(Pls. XIV and XV.)

As far as I know, these parts of the proboscis have not been fully described in the Hippoboscidae. The end view of the labellum shown in Fig. 10 of Müggenburg's paper is incorrect, whilst Massonnat does not refer to the prestomal teeth at all. The structure of the distal end of the labellum and the prestomal teeth was first described by Patton and Cragg (1913, p. 64) in *Hippobosca maculata* Leach, as follows: "There are five prestomal teeth on each side, arranged in a radiate manner, the middle and largest tooth being directed straight forwards. Its distal end is bifid, with two equal arms, while in the teeth on either side of it the arm next the middle line is the largest. The proximal ends of the teeth are rounded and are fitted into the distal end of the labial gutter. The external set of teeth are flattened and typically serrated with points directed upwards towards the head." Although I have not examined this structure in *Hippobosca maculata*, I doubt the accuracy of the description of it given by Patton and Cragg, and believe that the structure and arrangement of the prestomal teeth in the species studied by them will prove to be the same as in the labellum of the species described below.

The structure of the labellum in the Hippoboscidae is much more simple than that of *Glossina*, although in the former this part of the proboscis attains the higher degree of adaptation for penetration into the skin of the host. The labellum represents a deep gutter with thick chitinised walls the attachments of which to the labial gutter and the theca have already been described.

The position of the labial gutter with regard to the labellum varies: in the proximal part it lies above the labellum (Pl. XIV, figs. 34 and 37; Pl. XV, figs. 46 and 50), whereas distally it gradually becomes enclosed by the sides of the labellum until, in the anterior part of the latter, it is completely surrounded by it, occupying its centre (Pl. XIV, fig. 31; Pl. XV, figs. 44 and 49). The anterior border of the labial gutter is connected with that of the labellum by a membrane which forms the sides of the circular opening into the food canal. This opening, or prestomum, is surrounded by an outer and an inner set of prestomal teeth which have a radial arrangement and are movably attached to the membrane (Pl. XIV, figs. 31 and 32; Pl. XV, fig. 49).

In the outer set there are ten teeth, which are all of equal size and slightly curved. According to their position and structure they can be divided into two dorsal (*D.T.*), two ventral (*V.T.*) and six lateral (*L.T.*) teeth. All the lateral teeth are similar in structure and take the form of more or less cylindrical rods the bases of which are slightly broader than the middle parts. They terminate by two distinct denticles, at the bases of which and lying between them is a third very short denticle. The dorsal and ventral teeth differ from

the lateral ones in the presence of a very distinct denticle at the base of their dorsal surface. The teeth of the outer set are attached to the membrane between the anterior border of the labellum and that of the labial gutter, the spaces between them being of equal length.

The inner set has six prestomal teeth (*I.T.*) attached to the membrane at the anterior border of the labial gutter. All these teeth are of equal size, are flat and triangular in form and their free ends have each three distinct denticles, the middle denticle being much more developed than the other two, which are very small.

In addition to the prestomal teeth there are two serrated ridges (*S.R.*) which are situated on the dorsal borders of the labellum in its anterior part. The teeth of the serrated ridge (external set of teeth of Patton and Cragg, 1913) are very small, flat and bent backwards towards the head.

On the lateral surface of the labellum are two spines (*Sp.*) projecting from the shallow round pits, which have a similar structure to those described in the labellum of *Glossina*. Spines of the same type are arranged in rows on each side of the anterior border of the labellum. They alternate with stumpy tubules (*Tu.*) which bear peg-like projections on their dilated free ends. Between these rows of spines and the gustatory sensillae (*G.S.*) are a number of very small and almost inconspicuous rods (*Rd.*) which arise from the membrane. The gustatory sensillae are very similar in structure to those on the labellum of *Musca* and *Calliphora*. They are of two sizes: small and large. The large sensillae are arranged in two rows on the middle of the membrane between the spines and the prestomal teeth, each row consisting of four, and the small sensillae are two in number and are attached between the teeth of the outer set on each side.

The walls of the labellum and the labial gutter forming the cavity in the labellum are covered with a single layer of hypodermic cells, and do not carry any muscles, the space being filled with epithelial cells, tracheae and nerves.

The structure of the labellum in the Hippoboscidae is slightly variable. The type of prestomal teeth and their arrangement are similar in all the species which have been studied. In *Hippobosca equina* the labellum is more or less cylindrical, the prestomal teeth are very stout and much more chitinised than in *Lynchia maura*, in which the labellum is slightly conical and the prestomal teeth small in size. In *Melophagus ovinus* the labellum is of a regular cylindrical shape and longer than in the two preceding species, and is armed with narrow but long prestomal teeth.

The Muscles of the Haustellum and the Action of the Labellum and the Prestomal Teeth.

(Pl. XIV, fig. 43; Pl. XV, fig. 52.)

The inner surface of the theca and the labial gutter is covered with a single layer of hypodermic cells. After entering the bulb the labial nerve (*N.*) and the tracheae (*Tr.*) pass under the labial gutter through the cavity of the

haustellum into the labellum. The cavity of the narrow part of the haustellum is devoid of muscles, the bulbous part alone being provided with muscles which are arranged in the same number of sets as in the Muscidae.

The *retractors of the labellum* (*R.Lbl.*) correspond to the retractors of the furca in *Musca* (Hewitt, 1914) and to the M. longitudinales ventrales labii in lower Schizophora (Frey, 1921). This pair of muscles arises from the postero-lateral part of the ventral half of the bulb, their tendons uniting into one very strong common tendon (*T.*) which passes through the narrow part of the haustellum and is attached to the postero-lateral part of the labellum. The contraction of these muscles pulls the labellum towards the head, everting the membrane and the prestomal teeth.

The *protractors of the labial gutter* (*P.L.G.*) correspond to the dilators of the labium-hypopharynx in *Musca* (Hewitt, 1914) and to the M. transversales labii in lower Schizophora (Frey, 1921). These muscles arise from the posterior part of the labial gutter, are directed obliquely and anteriorly and occupy the middle part of the cavity of the bulb, their short hyaline tendons (*Ts.*) being inserted into the theca between its bulbous and narrow parts. When these muscles contract they protract the labial gutter, causing the membrane and the prestomal teeth to evert still more.

The *retractors of the labial gutter* (*R.L.G.*) correspond to the retractors of the discal sclerites in *Musca* (Hewitt, 1914) and to the M. longitudinales dorsales labii in lower Schizophora (Frey, 1921). These muscles arise from the upper postero-lateral parts of the dorsal half of the bulb and are inserted into the lateral processes of the labial gutter. They serve to retract the labial gutter and invert the membrane and the prestomal teeth.

The structure of the labellum and the arrangement of the muscles in the bulbous part show that the cutting action of the prestomal teeth depends upon the movements of the labellum and the protraction and retraction of the labial gutter. It must be pointed out that during the action of the protractors and retractors of the labial gutter the theca is capable of moving forwards and backwards along the gutter on account of the elasticity of the thin wall of the bulb, whereas the labial gutter is immovable in respect of the hyoid. These movements cause the membrane of the distal end of the labellum to become alternately everted and inverted, the prestomal teeth attached to this membrane performing protrusible divergent movements. When the proboscis is at rest the membrane and the prestomal teeth are inverted and the opening into the food canal is closed by the teeth. In the reverse condition the teeth of the outer set are directed sideways, their dentated ends pointing towards the head, as shown in Pl. XIV, fig. 37, Pl. XV, figs. 50 and 51, whilst the teeth of the inner set protrude from the prestomum, their dentated ends being directed to the sides.

The method of feeding was observed under the binocular microscope at a magnification of one hundred on five sheep-keds (*Melophagus ovinus*) which were placed on the upper part of my forearm. Before the ked starts feeding

it must attach itself to the host. As the human skin is very poor in hair much time is required before the ked becomes fixed. The ked then protrudes the haustellum between the maxillary palps, applying the end of the labellum to different points of the skin, apparently in search of a suitable spot for piercing. When such a spot is selected the insect pushes its proboscis against the skin with such force that the middle part of the haustellum bends into a bow. Sometimes in piercing the ked twists the haustellum as one twists an awl. Immediately afterwards there can be observed forward and backward movements in the labellum, the distal part of the proboscis rapidly penetrating into the skin. The haustellum penetrates into the skin as far as two-thirds of its length. The appearance of a red spot under the vertex indicates the blood being sucked through the pharynx into the oesophagus.

All the keds fed under observation were gorged in about ten minutes after the appearance of the red spot in the region of the oesophagus. It has also been observed that forcible removal of the feeding ked involves the risk of tearing off the haustellum, as it is fixed by the everted prestomal teeth to the bottom of the hole formed in the skin.

I felt no irritation during the feeding of the sheep-keds but four or five days later small yellowish papules made their appearance. Each had a very minute opening through which serum exuded. The irritation caused by these papules is not very severe but they heal very slowly, disappearing three or four weeks after the feeding of the insects.

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EXPLANATION OF PLATES XI–XV.

PLATE XI.

Figs. 1–9. *Lynchia maura* Bigot.

Fig. 1. Dorsal view of head. Second segment of left antenna removed to show antennal pit (*A.P.*) and first antennal segment (1 *S.*). ×45.

Fig. 2. Ventral view of head, showing median membranous part and position of bulb. ×45.

Fig. 3. Posterior view of head. ×45.

Fig. 4. Scales of ptilinal membrane. ×600.

Fig. 5. Scales of medio vertex. ×600.

Fig. 6. Spines from inner side of second antennal segment. ×600.

Fig. 7. Left antenna. Dotted lines indicate position of third antennal segment (3 *S.*). ×110.

Fig. 8. Third antennal segment with arista (*A.*) and olfactory pits (*O.P.*). ×150.

Fig. 9. Part of inner view of head, showing position of ptilinum (*Pt.*) and ocular sclerite (*O.S.*). ×45.

PLATE XII.

Figs. 10–20. *Melophagus ovinus* L.

Fig. 10. Dorsal view of head. Second segment of right antenna removed to show antennal pit (*A.P.*) and first antennal segment (1 *S.*). ×45.

Fig. 11. Ventral view of head, showing median membranous part and position of bulb. ×45.

Fig. 12. Inner view of head, showing position of antennal pit (*A.P.*) with first antennal segment (1 *S.*), ptilinum (*Pt.*) and position of torma of fulcrum (*To.*). ×45.

Fig. 13. Posterior view of head, showing occipital foramen (*Oc.F.*) and attachment of latero-cervical sclerite (*Lc.*). ×45.

Fig. 14. Scales of mediovertex. ×600.

Fig. 15. Scales of ptilinal membrane. ×600.

Fig. 16. Spines from inner wall of second antennal segment. ×750.

Fig. 17. Second antennal segment. Dotted lines indicate position of third antennal segment (3 *S*). × 110.

Fig. 18. Third antennal segment with arista (*A.*) and olfactory pit (*O.P.*). × 225.

Fig. 19. Sensilla from olfactory pit. × 750.

Fig. 20. Toothed process of second antennal segment. × 375.

PLATE XIII.

Figs. 21–26. *Lynchia maura* Bigot.

Fig. 21. Labrum-epipharynx showing attachment of stipites (*St.*) and rostrum membrane (*R.M.*). × 100.

Fig. 22. Dorsal view of bulb: labrum-epipharynx removed to expose labial gutter (*L.G.*) and hypopharynx (*H.*). × 100.

Fig. 23. Torma. × 100.

Fig. 24. Bristles of mulberry-shaped organ on membrane of bulb. × 750.

Fig. 25. Pharynx, showing median plate (*M.P.*) and attachment of lateral plates (*L.P.*). × 100.

Fig. 26. Proximal part of haustellum; theca removed to show labial gutter (*L.G.*) hypopharynx (*H.*), labrum-epipharynx (*Lep.*) and hyoid (*Hy*). × 150.

Figs. 27–30. *Melophagus ovinus* L.

Fig. 27. Labrum-epipharynx, showing attachment of stipites (*St.*). × 100.

Fig. 28. Pharynx, showing median plate (*M.P.*), attachment of lateral plates (*L.P.*) and hyoid (*Hy.*). × 110.

Fig. 29. Dorsal view of bulb; labrum-epipharynx removed to expose labial gutter (*L.G.*) and hypopharynx (*H.*). × 100.

Fig. 30. Ventral view of torma (*To.*) and attachment of lateral plates of fulcrum (*L.P.*). × 175.

PLATE XIV.

Fig. 31. *Hippobosca equina* L. End view of labellum (slightly flattened), showing two sets of prestomal teeth completely everted. × 750.

Figs. 32–43. *Lynchia maura* Bigot.

Fig. 32. End view of labellum, showing two sets of prestomal teeth inverted. × 750.

Fig. 33. Oblique transverse section through distal part of labellum. × 565.

Fig. 34. Transverse section through proximal part of labellum. × 565.

Fig. 35. Transverse section through distal part of haustellum. × 565.

Fig. 36. Transverse section through middle part of haustellum. × 565.

Fig. 37. Lateral view of labellum and distal part of haustellum. Prestomal teeth completely everted. Dotted lines indicate position of labrum-epipharynx (*Lep.*), hypopharynx (*H.*), tendon (*T.*) and ventral limit of labial gutter (*L.G.*). × 600.

Fig. 38. Longitudinal section of salivary duct showing valve (*Sl.V.*) and its muscle (*Sl.V.M.*). × 450.

Fig. 39. Transverse section through maxillary palp. × 450.

Fig. 40. Transverse section through proximal part of bulb, showing mulberry-shaped organ (*M.O.*). Here epipharynx and labrum become fused. Hypopharynx completely fused with labial gutter. × 450.

Fig. 41. Transverse section of hyoid and salivary duct. × 450.

Fig. 42. Transverse section of labial gutter. Here hypopharynx becomes free from labial gutter. × 450.

Fig. 43. Transverse section through middle of bulb, showing arrangement of muscles. × 450.

PLATE XV.

Figs. 44–56. *Melophagus ovinus* L.

Fig. 44. Transverse section through distal part of labellum. × 565.

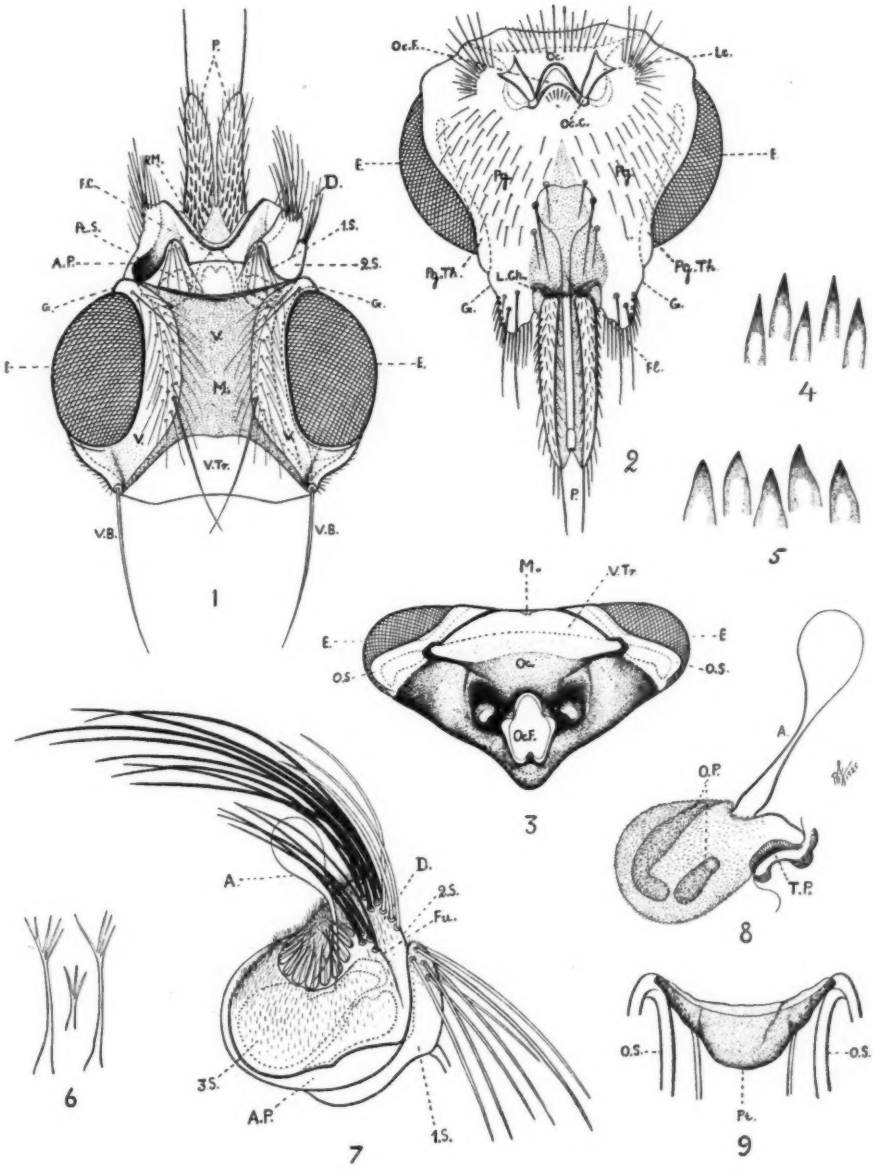
Fig. 45. Transverse section through middle of labellum. × 565.

Fig. 46. Transverse section through proximal part of labellum. × 565.

Fig. 47. Transverse section through distal part of haustellum. × 565.

Fig. 48. Transverse section through middle of haustellum. × 565.

Fig. 49. End view of labellum, showing prestomal teeth nearly everted. × 750. Three types of prestomal teeth (*D.T.*, *L.T.*, *I.T.*). × 1500.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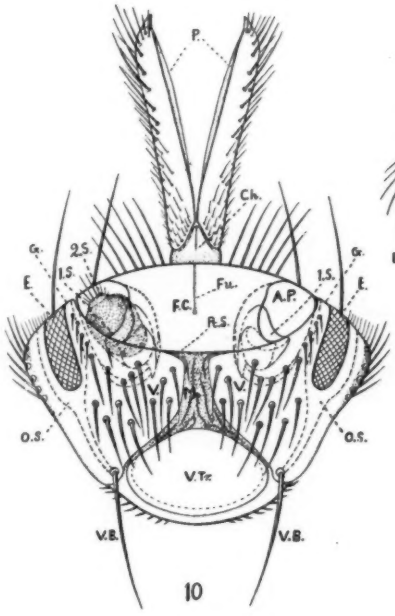

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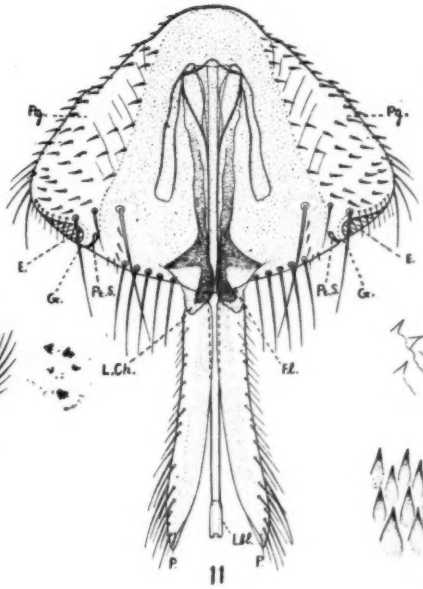

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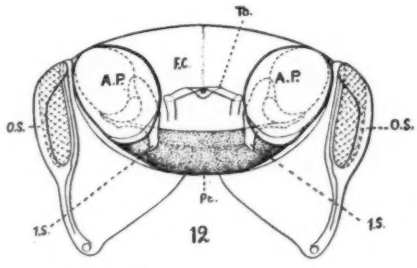

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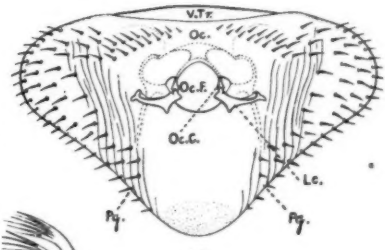

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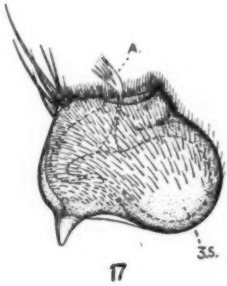

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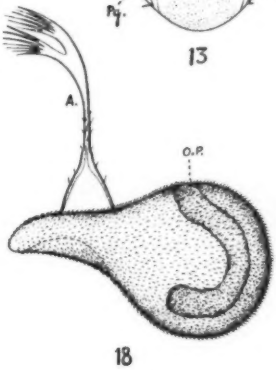

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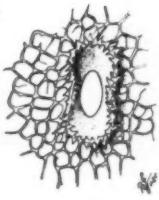

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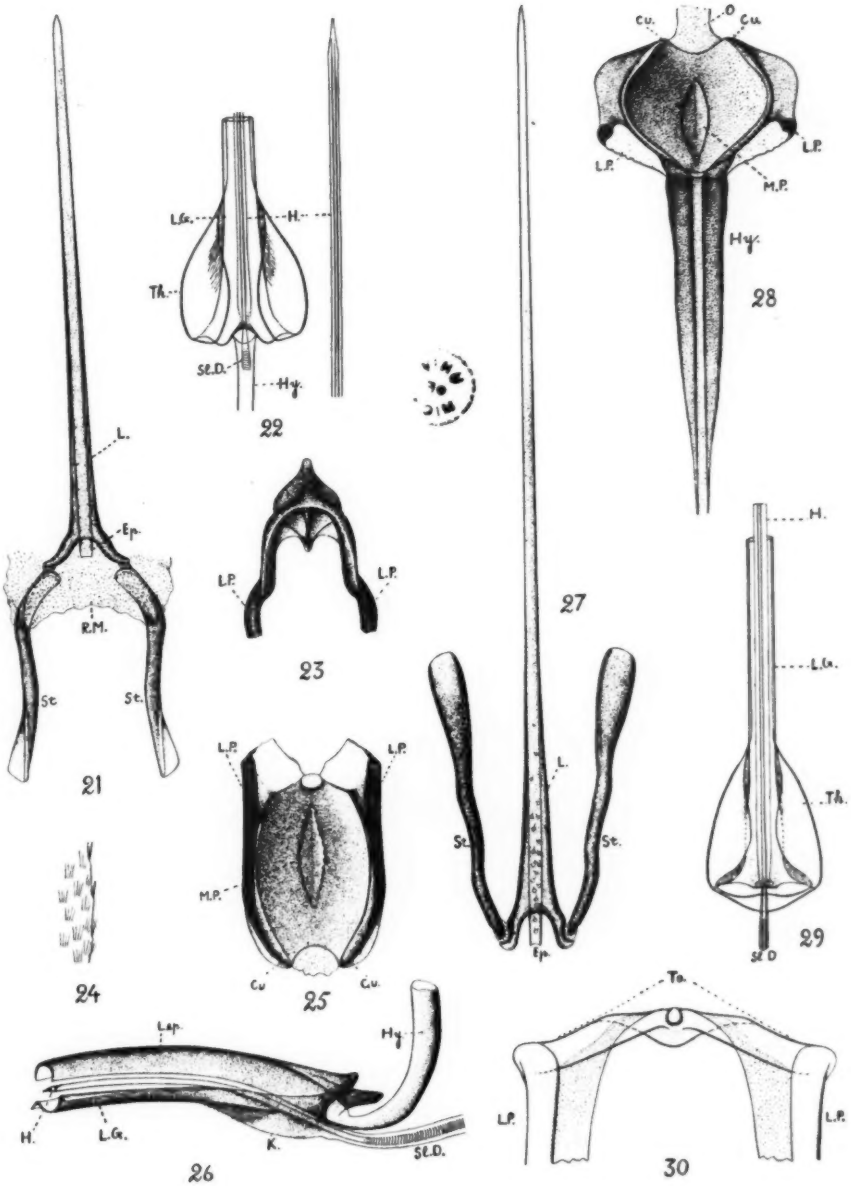
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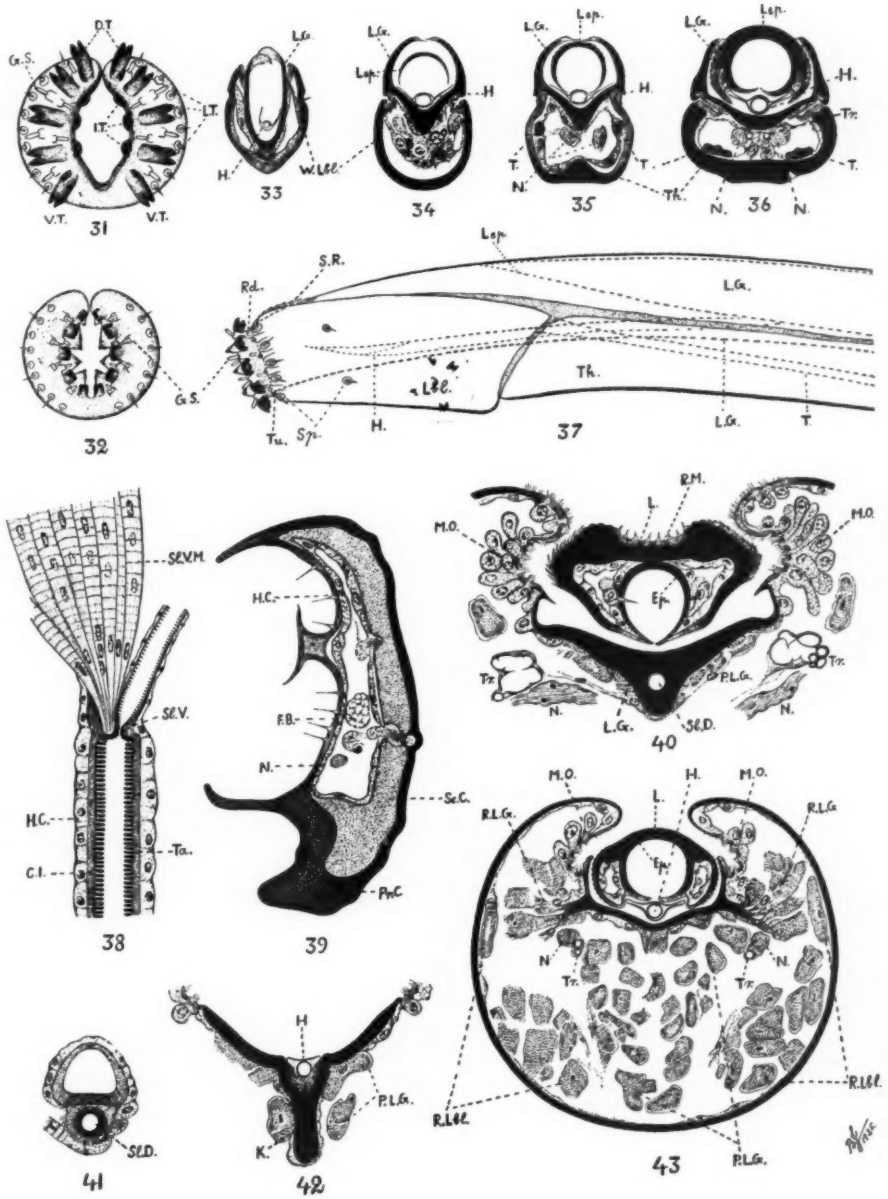
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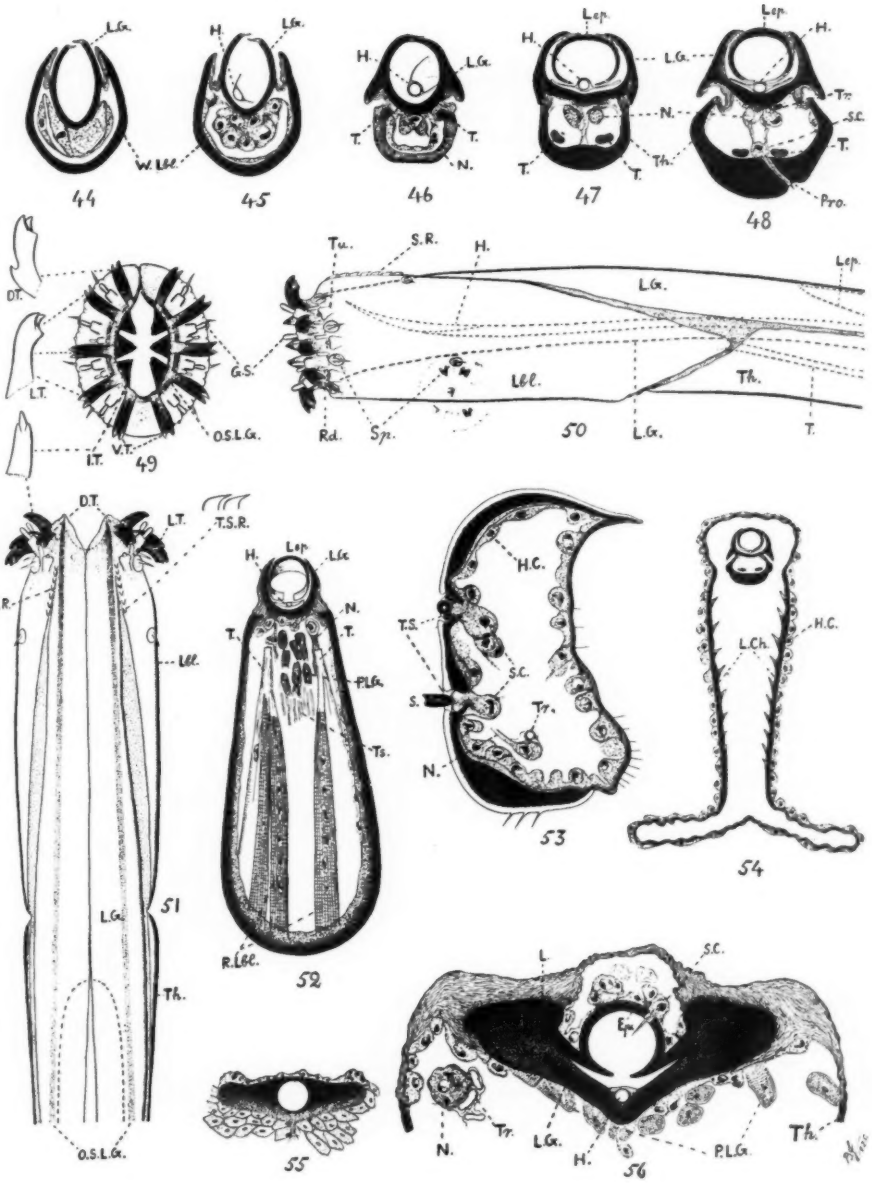
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Fig. 50. Lateral view of labellum and distal part of haustellum. Prestomal teeth completely everted. Dotted lines indicate position of labrum-epipharynx (*Lep.*), hypopharynx (*H.*), tendon (*T.*) and ventral limit of labial gutter (*L.G.*). ×600.

Fig. 51. Dorsal view of labellum and distal part of haustellum. Prestomal teeth completely everted; labial gutter protracted. Dotted line indicates position of labrum-epipharynx. ×600. Two teeth of serrated edge of labellum. ×1500.

Fig. 52. Oblique transverse section through anterior part of bulb, showing attachment of retractors of labellum (*R.Lbl.*) to tendon (*T.*). ×225.

Fig. 53. Transverse section through maxillary palp. ×450.

Fig. 54. Transverse section through rostrum membrane, showing position of haustellum and lateral chitinised parts (*L.C.*). ×185.

Fig. 55. Transverse section of hyoid. ×225.

Fig. 56. Transverse section through base of bulb. Here epipharynx (*Ep.*) and labrum (*L.*) become fused; hypopharynx (*H.*) becomes separated from labial gutter (*L.G.*) and theca connected with labial gutter and labrum by filamentous membrane. ×450.

EXPLANATION OF REFERENCE LETTERING.

A. Arista
A.P. Antennal pit
A.S. Air sac
Ch. Chitinised part of rostrum membrane
C.I. Chitinous intima
Cu. Cornu
D. Dorsal prolongation of second antennal segment
D.F. Depressor of fulcrum
D.Ph. Dilator of pharynx
D.T. Dorsal tooth
E. Compound eye
E.St. Elevator of stipes
E.Hy. Elevator of hyoid
Ep. Epipharynx
F. Fulcrum
F.B. Fat-body
F.C. Fronto-clypeus
Fl. Flap
Fu. Furrow
G. Gena
G.S. Gustatory sensilla
H. Hypopharynx
H.C. Hypodermic cell
Hy. Hyoid
I.T. Internal tooth
K. Keel of labial gutter
L. Labrum
Lbl. Labellum
Lc. Latero-cervical sclerite.
L.Ch. Lateral chitinised part
Lep. Labrum-epipharynx
L.G. Labial gutter
L.P. Lateral plate of fulcrum
L.T. Lateral tooth
M. Mediovertex
M.O. Mulberry-shaped organ
M.P. Median plate
N. Nerve
O. Oesophagus
Oc. Occiput
Oc.C. Occipital condyle
Oc.F. Occipital foramen
O.P. Olfactory pit
O.S. Ocular sclerite
O.S.L.G. Optical section of labial gutter
P. Maxillary palp
Pg. Postgena
Pg.Th. Postgenal thickening
P.H. Protractor of haustellum
Ph. Pharynx
P.L.G. Protractor of labial gutter
Pr.C. Primary cuticula
Pro. Pore
Pt. Ptilinum
Pt.S. Ptilinal suture
R.St. Retractor of stipes
Rd. Rod
R.H. Retractor of haustellum
R.Lbl. Retractor of labellum
R.L.G. Retractor of labial gutter
R.M. Rostrum membrane
R.R. Retractor of rostrum
S. Seta
Sb.O.G. Suboesophageal ganglion
S.C. Sensory cell
Sc.C. Secondary cuticula
Sl.D. Salivary duct
Sl.V. Salivary valve.
Sl.V.M. Salivary valve muscle.
S.O. Sphincter of oesophagus
Sp. Spine
Sp.O.G. Supraoesophageal ganglion
S.R. Serrated ridge of labellum
St. Stipes
T. Tendon
Ta. Taenidia
Th. Theca
To. Torma
T.P. Toothed process
Tr. Trachea
T.S. Theca of seta
Ts. Tendons
T.S.R. Teeth of serrated ridge
Tu. Stumped tubules
V. Vertex
V.B. Vertical bristle
V.T. Ventral tooth
V.Tr. Vertical triangle
W.Lbl. Wall of labellum
1 *S.* First antennal segment
2 *S.* Second antennal segment
3 *S.* Third antennal segment

(*MS. received for publication* 2. II. 1926.—Ed.)

ON THE GENUS *CRATAERINA*, VON OLF., AND ITS ALLIES (DIPTERA PUPIPARA—FAMILY HIPPOBOSCIDAE), WITH DESCRIPTIONS OF NEW SPECIES[1].

By Major E. E. AUSTEN, D.S.O.

(With Plate XVI and 1 Text-figure.)

Among the bird-parasites included in the family Hippoboscidae, are four small, closely connected genera, in all of which the wings exhibit more or less extensive reduction, and are apparently useless for the purpose of flight. In various parts of the world species belonging to one or other of three of these genera, namely *Crataerina*, v. Olf., *Myophthiria*, Rond., and *Brachypteromyia*, Will., infest Swifts (*Micropus*, *Collocalia*, etc.) and their nests; while the solitary representative of the fourth genus *Stenepteryx*, Leach, at present known is similarly parasitic on the House-Martin (*Delichon urbica*, L.), which, though in no wise related, is closely associated with the Common Swift in the popular mind.

Key to the Four Genera of Hippoboscidae mentioned above.

Claws tridentate; wings more or less reduced.

1 (2). Ocelli present, or entirely or partly absent, sometimes wholly or partly represented by depressions; wings entirely narrow and ribbon-like *Stenepteryx*, Leach.

2 (1). Ocelli always wanting; wings never entirely ribbon-like.

3 (4). Wings minute and disciform, about as long as broad, and scarcely extending beyond base of abdomen . . . *Brachypteromyia*, Will.

4 (3). Wings reaching at least to middle of abdomen, and often extending far beyond its tip.

5 (6). Wings blunt and short, extending to middle of abdomen or beyond but not reaching its tip, with only two (in one or more species three?) longitudinal veins behind costa . . . . . *Myophthiria*, Rond.

6 (5). Wings usually extending beyond, often far beyond tip of abdomen, and, except in one or two species in which they are blunt and abbreviated, pointed or attenuate at the tips, in several cases approximately the distal third being narrow and ribbon-like; longitudinal veins behind costa normal in number. . . . . *Crataerina*, v. Olf.

[1] The whole of the material, including types of new species, upon which the following paper is based, is in the British Museum (Natural History).

For information as to occurrence and correct nomenclature of hosts, the author is indebted to the kindness of his colleague Mr N. B. Kinnear.

Genus **Stenepteryx**, Leach.

Leach (1818), *Mem. Wern. Nat. Hist. Soc.* II. 549, 551.

Genotype: *Hippobosca hirundinis*, L.

Apart from the characters furnished by the wings, this genus is usually stated to be distinguished from *Crataerina* by the presence of ocelli. As pointed out, however, by Massonat (1909, *Ann. Univ. Lyon*, Nouv. Sér. I, Fasc. 28, pp. 40, 41), these structures in *Stenepteryx* are in process of disappearance. In certain specimens of *S. hirundinis* no trace of simple eyes can be perceived, even under fairly high magnification; in others, although the anterior ocellus may be present, the remaining two ocelli are apparently represented merely by pits. As regards the *wings*, the loss of part of the hinder portion of the wing seen in *Crataerina* has progressed still further in the case of *Stenepteryx*, in which the wings are reduced to narrow, ribbon-like strips, at most some three times broader at their widest part than at the tips. A portion of the thickened part of the costa, from a little beyond the base to about the level of *r–m* (the anterior transverse vein), is fringed with long hairs similar to those present on the costal margin in *Crataerina*.

As already indicated, the only species of *Stenepteryx* hitherto described is the genotype:

Stenepteryx hirundinis, L.

The specimens of both sexes forming the small Museum series of *S. hirundinis* are chiefly British, the British examples being, with two (Welsh) exceptions, from various localities in England. The dates of capture range from June 20th to October 14th, and most of the specimens are stated to have been obtained either on House-Martins or in their nests; one individual is said, perhaps erroneously, to have been found on a "*Swallow*," which was picked up dead. The General Collection of Diptera includes, besides examples of *S. hirundinis* from Norway, a single (♂) specimen from India (W. Himalayas, Thandiani, 9000 ft., 3. vii. 1907, *Major H. A. F. Macgrath*, "Parasitic on House-Martin"), which, except in being slightly smaller, does not present any noticeable differences from the typical form. There is also a ♀ without locality, but bearing the label "Hirundinis" in Leach's handwriting, and therefore presumably more than a century old.

Genus **Crataerina**[1], von Olfers.

Von Olfer's *De Vegetativis et Animatis Corporibus in Corporibus Animatis*

[1] The spelling here adopted is that used by the author of the genus, who states that the name is derived from the Greek κράταιρῖνος (*hard-shelled*). Bezzi (1905, *Katalog der Paläarktischen Dipteren*, IV. 280), who quite unnecessarily introduces *Crataerrhina* as an emendation, misquotes the original spelling as *Crataerhina*, a form previously adopted however by Speiser (1900, *Ann. Mus. Civ. Stor. Nat. Genov.* (Ser. 2*a*), XX. 555).

Bezzi (*loc. cit.*) also gives the date of von Olfers's work incorrectly as 1805, and (following Hagen—see below) the place of publication as "Goettingae."

Reperiundis Commentarius, Pars I, p. 101 (Berolini: In Taberna Libraria Maureriana, 1816)[1].

Genotype: *Ornithomyia pallida*, Latr. (*Crataerina lonchoptera*, v. Olf., *op. cit.* p. 102).

In this genus, although the posterior half of the wing shows more or less extensive reduction, the full number of longitudinal veins is always present. The three species of *Crataerina* (*C. pallida*, Latr., *C. melbae*, Rond., and *C. sibiriana*, Gimm.) hitherto known are all Palaearctic. The four new species described below include one species which has been found in the Mediterranean Sub-Region; another which has been met with both in the Mediterranean Sub-Region and the Ethiopian Region; an Oriental species; and a fourth species, of which the available representatives are partly from the Palaearctic, and partly from the Oriental Region.

Synoptic Table.

The six species described or recorded below are mutually distinguishable as follows:

1 (4). Wings either pointed or blunt at the tips; in the former case not more than the distal fourth attenuate.
2 (3). Wings pointed at the tips . . . . . *pallida*, Latr.
3 (2). Wings blunt at the tips (and small) . . . *obtusipennis*, sp. n.
4 (1). Wings, besides being usually long, remarkably narrowed at the tips; approximately the distal third strongly attenuate.
5 (6). Second basal cell normally divided into two by a supernumerary transverse vein . . . . . *melbae*, Rond.
6 (5). Second basal cell not divided.
7 (10). Second basal cell about twice as long as anal cell.
8 (9). Wings relatively broad a little beyond level of *r–m* (anterior transverse vein), hind margin with a rounded angle, or elbow, below tip of *2dA* (sixth longitudinal vein), then concave . . . *propinqua*, sp. n.

[1] With reference to the title, and place and date of publication of this excessively rare little book, it is perhaps worth while to quote the following note from the present writer's *Illustrations of African Blood-Sucking Flies* (London: Printed by Order of The Trustees of the British Museum, 1909), pp. 176, 177, a work which itself has been out of print for many years.—"As given by Hagen (*Bibliotheca Entomologica*, II. 20 (Leipzig: Verlag von Wilhelm Engelmann, 1863)) the title of this work is 'De vegetativis et animatis corporibus in corpore animato reperiundis, Pars I.' Hagen also gives the place and date of publication as 'Goettingae, 1815.' In none of the catalogues that I have been able to consult, however, is there any mention of an 1815 (Göttingen) edition; the 1816 (Berlin) edition alone is mentioned, and the title quoted is the same as that given above, which is taken from the Museum copy, formerly in the library of Sir Joseph Banks. Wiedemann (*Aussereuropäische zweiflügelige Insekten*, II. 604, note, 1830) quotes the 1816 edition.—Since the foregoing was written, I have received a most courteous letter from Dr F. Reicke, of the Königliche Universitäts-Bibliothek, Göttingen, informing me, in reply to an enquiry, that a copy of the 1816 edition of von Olfers's work is the only one contained in that institution; and that, in spite of an exhaustive search, he has been unable to discover any evidence of the existence of an 1815 (Göttingen) edition."

Crataerina pallida, Latr., Female.

(Natural size indicated by the line below.)

9 (8). Wings not especially broad a little beyond level of *r–m*, hind margin with no elbow-like prominence below tip of *2dA*, but convex to beyond level of termination of thickened portion of costa
acutipennis, sp. n.

10 (7). Second basal cell about three times as long as anal cell
longipennis, sp. n.

Crataerina pallida, Latr. (Pl. XVI, Fig. 1 *b*).

Ornithomyia pallida, Latreille (1812), *Encycl. Méthod.* VIII. 544.

This species, which is a parasite of the Common Swift (*Micropus apus*, L.)[1], and in Southern Europe likewise found in the nests of the Alpine Swift (*Micropus melba*, L.), is the only representative of its genus that occurs in the British islands. The length of the ♂ is about 6 mm.; that of the pregnant ♀ may be as much as 7·6 mm., while the abdomen of the latter across its widest part may measure 6·2 mm. Pregnant ♀♀ of *C. pallida* preserved in spirit show a single small transverse plate of ochraceous-tawny chitin, deeply indented posteriorly, on the upper surface of the abdomen, in the middle line in front of the apical plates. A collector's field-note on eight of these flies (all pregnant ♀♀) taken on a young Swift, at Cheadle, Staffordshire, 24. vii. 1916 (*J. Masefield*), describes the abdomen in life as "bright bluish-green."

As may be seen on comparing Pl. XVI and Fig. 1 *b*, the degree of acuity exhibited by the tip of the wing in *C. pallida* is subject to a considerable amount of individual variation, although in pinned specimens, as the result of accidental damage, this feature is often obscured. It is possible that the type of Leach's *Oxypterum kirbyanum*[2] (1818, *Mem. Wern. Nat. Hist. Soc.* II. 563, Tab. XXV, Figs. 15, 16), which was described from a solitary specimen taken in Suffolk by the Rev. William Kirby, may thus owe the bluntness of its wings, at least in part, to *post mortem* injury. On referring to Pl. XVI and Fig. 1 *b*, it will be seen that in *C. pallida*, as in all of its known congeners, the thickened portion of the costa is fringed with long hairs, the longest hairs in the case of *C. pallida* being between the humeral transverse vein and the junction of R_{2+3} (second longitudinal vein) with the costa, although one or more fairly long hairs are often visible just before the tip of the thickened portion of the latter. The longer hairs forming the costal fringe in *C. pallida* are apparently 21 or 22 in number in the case of the ♂, and from 21 to 32 in that of the ♀, but, since they are very liable to be rubbed off, in pinned specimens part of them is usually missing.

The National Collection includes a number of examples of both sexes of *C. pallida*, taken, chiefly in Swifts' *nests*, in various English counties from Somerset to Staffordshire. In one instance a specimen was found by a collector on his coat-sleeve indoors, while another was taken on a cottage

[1] *Apud* Bezzi (1905, *Kat. Pal. Dipt.* IV. 281, note *), the Chimney-Swallow (*Hirundo rustica*, L.) is also a host of *C. pallida*, but no support to this statement is afforded by data attached to specimens in the Museum.

[2] Treated as a variety of *C. pallida*, Latr., by Bezzi (*loc. cit.*).

window-sill, below an empty Swift's nest. As bearing on the possibility that *C. pallida* may act as carrier of some micro-organism pathogenic to its hosts, it is perhaps worth noting that on one occasion (Wimbledon, 14. vii. 1922) several specimens—at least five (all ♀♀)—were found on a dying Swift; and that another example (likewise a ♀) was caught on one of these birds, which was clinging to a gutter (Hanwell, 8. viii. 1895) in a half-dead state[1].

Apart from two old specimens without precise data, the only Continental individuals of *C. pallida* in the Museum are from Switzerland, and consist of: seven ♂♂ from Geneva, May, 1911 (*A. Ghidini*), on *Micropus apus*, L.; and a ♂ and two ♀♀ from Berne, 23. vi. 1887 (*Rev. H. A. Macpherson*). The collector's note received with the latter flies is as follows: "Infesting the inner lining of nests of the Alpine Swift (*Micropus melba*, L.), in the belfry of the Domkirk at Berne. The infested nests were on the beams of the belfry, in almost total darkness. When disturbed, the parasites run with agility, but also seek protection by crouching motionless on the nest."

Apparently this species, if for any reason it should become separated from its natural hosts and their nests, and enter the living rooms of houses, may attack human beings. Thus, it is stated by Dr Erich Hesse (1920, *Zeitschr. f. wiss. Insektenbiol.* xvi. 33) that, during the summer of 1918, several cases occurred at Leipzig in which *C. pallida* "made itself very troublesome" to people in bed at night. Again, Dr Erwin Christeller (*t. cit.* 1924, xix. 103) has recorded a case in which he himself, while sitting at an open window in his house at Charlottenburg, at the beginning of September, 1923, was bitten on the back of the neck by a specimen of this fly, the bite feeling somewhat like that of a midge.

The fact that, in the case of three series of specimens of this species in the Museum, the individuals forming the series are all of the same sex, would seem to indicate a certain segregation of the sexes in nature—perhaps only at particular seasons.

So far as it is possible to judge from Guérin-Méneville's very inaccurate description and figure, there can be little doubt that Bezzi (*Kat. Pal. Dipt., loc. cit.*) is correct in treating *Anapera tangerii*, Guér. (1844, *Icon. Règne Anim.* iii. 556, Pl. 104, fig. 6), the type of which was obtained at Tangier, as identical with *Crataerina pallida*, Latr.

[1] It has recently been pointed out by Wülker (3. xi. 1925, "Zur Biologie der Lausfliegen der Vögel und ihrer Rolle als Protozoenüberträger," *Senckenbergiana*, vii. Heft 6, p. 229) that: "Dissemination of blood-parasites, by means of the species [*i.e.* those with reduced wings] that are closely attached to the nest and the avian host, can obviously occur only much more slowly and irregularly than when the intermediaries belong to genera such as *Ornithomyia*, *Lynchia*, etc., the species of which are rapid fliers, capable of passing easily to and fro between nests and hosts." In a note on the same page, Wülker mentions that Franchini (1923, *Bull. Soc. Path. exot.* T. 16) has recorded a few instances in which trypanosomes have been found in Italian Swifts—presumably *Micropus melba*, L.

Crataerina acutipennis, sp. n. (Fig. 1 *a*).

♂♀. Length, ♂ (four *dried* specimens) 4·4 to 5·6 mm., ♀ (five *dried* specimens —two non-gravid, three gravid) 5 to 7·4 mm.; width of head, ♂ 1·25 to 1·6 mm., ♀ 1·5 to 1·6 mm.; length of wing, ♂ 4·25 to 5·25 mm., ♀ 4·6 to 5·25 mm.; greatest width of wing, ♂ 1·25 to 1·5 mm., ♀ 1·2 to 1·4 mm.

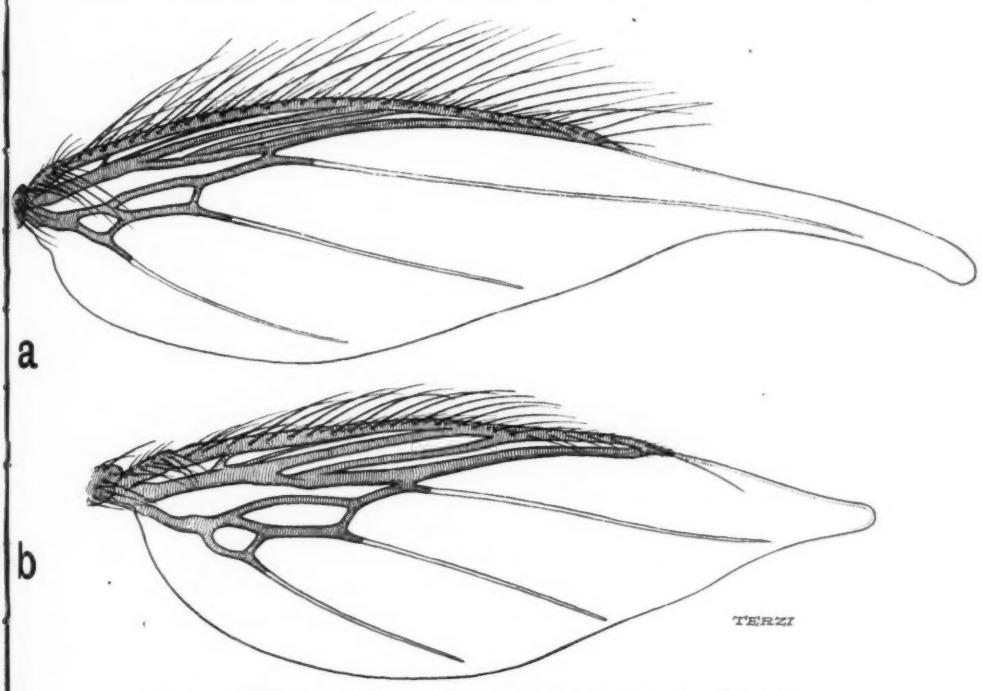

Fig. 1. *a*, Wing of *Crataerina acutipennis*, Austen; *b*, Wing of *C. pallida*, Latr. (Both *a* and *b* × 18.)

Differing from the European C. pallida, *Latr.*, inter alia *in the distal extremity of the wing being much more attenuate, with* M_{1+2} (fourth longitudinal vein) *extending as a rule further along it; and in the long hairs on the costa being in both sexes longer, finer and, at least apparently, more numerous* (cp. Fig. 1 *a* and *b*).

Head: hair clothing inner margins of orbits (parafrontals), and upper surface of *antennary prolongations*, long and fine (longer and finer than corresponding hair in case of *C. pallida*). *Abdomen:* small plates of chitin in median line on upper surface in ♀ (often difficult to distinguish in dried specimens), in front of apical plates, shining blackish-brown or mummy-brown[1]. *Wings:*

[1] For names and illustrations of colours used for descriptive purposes in the present paper, *see* Ridgway (1912), *Color Standards and Color Nomenclature* (Washington, D.C. Published by the Author).

attenuate portion of wing equal in length to about one-third of the whole, showing some slight individual variation as regards precise degree of acuity of tip, but in all cases conspicuously longer than in *C. pallida*, Latr., and narrower relatively to its length; normally chitinised veins less coarse (more slender) than in species mentioned.

MADEIRA; CANARY IS.; TRANSVAAL; NATAL.

Type of ♂, type of ♀ and two other ♀♀ (paratypes) from Pretoria, Transvaal, 22. vii. 1925 (*J. A. Macphail*: presented by the Imperial Bureau of Entomology); one ♂ from Durban, Natal, 20. xii. 1913 (*W. Butcher*), "from Swift's nest"; one ♂ from the Canary Is., Grand Canary, Firgas, near Las Palmas, 1625 ft., 30. iv. 1913 (*D. A. Bannerman*), on a Madeiran Black Swift (*Micropus unicolor unicolor*, Jardine); one ♂ from the Canary Is., Grand Canary, Las Palmas, 2. v. 1913 (*D. A. Bannerman*); one ♂ from the Canary Is., Fuerteventura, Toston, 7. v. 1913 (*D. A. Bannerman*), on a Brehm's Swift (*Micropus murinus brehmorum*, Hart.); one ♂, two ♀♀, from Madeira, 1852 (*J. Macgillivray*, H.M.S. "Herald"), on *Micropus unicolor unicolor*, Jardine.

The host of the specimen from Durban was perhaps *Micropus caffer*, Licht.

Crataerina obtusipennis, sp. n.

♀. Length (five *dried* specimens, including at least two which are gravid) 5·4 to 6·5 mm.; width of head 1·6 to 1·75 mm.; length of wing 2·25 to 2·6 mm.; greatest width of wing 1 mm. or just over 1 mm.

Distinguished from all of its known congeners, except C. sibiriana, *Gimm., by the shape of the wings, which are exceptionally small, blunt at the tips instead of pointed, and in form resemble those of certain species of* Myophthiria, *Rond.; further distinguishable by the coloration of the stouter veins, which are heavily chitinised, relatively coarse, and, except at the base of the wing, mummy-brown or blackish-brown.*

Head: antennae relatively (*e.g.* as compared with the condition in *C. pallida*, Latr.) rather close together at base, intervening area dark brown. General coloration of *head*, *thorax* and *legs* in *dried* condition tawny-olive or brownish-olive, but a greenish tinge sometimes still distinctly visible here and there in the legs. *Abdomen:* dorsum exhibiting, in addition to usual apical plates, three small reniform or inversely cordate plates of shining dark brown chitin in middle line. *Wings:* thickened portion of costa fringed with long, dark brown or blackish hair from its base to end of R_1 (first longitudinal vein), thence to tip apparently clothed exclusively with shorter hair; M_{1+2} (fourth longitudinal vein) often terminating abruptly about half-way (sometimes further, sometimes not so far) between *r–m* (anterior transverse vein) and wing-margin.

MONGOLIA and MALACCA (Penang).

Holotype and one paratype, Mongolia (precise locality unknown) (purchased from *Whitely*, 1875); three additional examples, Penang (ex coll. *J. C. Bowring*, 1863).

A possible host, occurring in both Mongolia and Malacca, is *Micropus pacificus*, Latham.

C. obtusipennis is perhaps identical with *C.* (*Anapera*) *sibiriana*, Gimmerthal (1847, *Progr. Riga*, p. 12; *Bull. Soc. Imp. des Nat. de Moscou*, xx. 2, p. 208), the type of which was obtained in Siberia. Gimmerthal's description, however, albeit extremely brief, contains discrepancies (the thorax is described as spotted with yellow above, while the third and fourth segments of the posterior tarsi are stated to be pale), so that, in default of a scrutiny of the type of *C. sibiriana*, certainty is impossible.

Crataerina longipennis, sp. n.

♂♀. Length, ♂ (one *dried* specimen) 5·75 mm., ♀ (one *dried* specimen) 5·5 mm.; width of head, ♂ 1·6 mm., ♀ 1·8 mm.; length of wing, ♂ 6·6 mm., ♀ 6·8 mm.; greatest width of wing, ♂ 1·75 mm., ♀ 1·8 mm.

Head in both sexes, viewed from above and exclusive of clypeus, antennary prolongations and mouth-parts, roughly circular in outline, about as broad as long; vertical triangle almost equilateral, its apex narrow, bluntly rounded at tip; wings long, fairly broad across level of r–m (*anterior transverse vein*), *distal third attenuate, posterior margin concave* (*at least not convex*) *at point at which* $M_3 + Cu_1$ (*fifth longitudinal vein*) *would join it if produced; legs long.*

Head: hair clothing inner margins of orbits, and upper surface of *antennary prolongations*, moderately fine; anterior two-thirds of vertical triangle in ♀ (dried specimen) shining mummy-brown; frontal stripe in both sexes rather narrow posteriorly, about half as broad as on its front margin. *Abdomen:* plates of chitin on upper surface, in front of apical plates, transversely elongate in both sexes, anterior plate relatively large in ♂ (in dried specimen occupying about one-third of total width of its segment). *Wings:* longer hairs on costa long and fine, from base to beyond level of anterior transverse vein, thence to tip of thickened portion shorter; M_{1+2} extending some two-thirds of way along attenuate portion of wing; second basal cell large, elongate, about three times as long as anal cell, and not divided by a transverse vein.

Burma: type of ♂, type of ♀, from N. Shan States, Gokteik Cave, 1000 ft., 10. v. 1915 (*C. G. Rogers*); from a Swift (*Micropus pacificus cooki*, Har.) picked up dead in cave.

From *Crataerina acutipennis*, Austen, the species just described is distinguishable in both sexes by the head being wider, the wings longer, as well as wider across the widest part, and the legs, notably femora and tibiae, longer. *Crataerina longipennis* may similarly be distinguished from the South European *C. melbae*, Rond., by the middle portion of the wing being distinctly narrower and its hind margin not convex, and by the second basal cell being larger and not transversely divided.

Crataerina propinqua, sp. n.

♀. Length (one dried specimen) 6 mm.; width of head 1·75 mm.; length of wing 6·6 mm.; greatest width of wing 2 mm.

Closely allied to and resembling foregoing species (C. longipennis, *Austen*), *but distinguishable by apex of vertical triangle being broad and blunt; by wing at its widest part being slightly but perceptibly broader, and having on its hind margin a distinct rounded angle, or elbow, below tip of* 2dA (*sixth longitudinal vein*); *and by second basal cell being small, narrow, and only twice as long as anal cell.—Wings long, rather more than distal third strongly attenuate.*

Head: frontal stripe short, and relatively broad in proportion to its length; vertical triangle (in dried specimen) with a mummy-brown median blotch between base and rounded apex. *Abdomen:* only one small transverse plate of chitin on upper surface, in front of apical plates, distinguishable (at least in case of type). *Wings:* second basal cell not transversely divided; posterior margin beyond elbow concave; M_{1+2} extending to about 0·6 mm. from extreme tip of wing (at least in case of type). *Legs* as in *C. longipennis*, Austen.

Syria, 1863–64, "parasitic on Swifts" (the late *B. T. Lowne*: ex coll. W. W. Saunders).

Although at the first glance liable to be confused with *C. longipennis*, this species can readily be recognised by means of the distinctive structural details mentioned above.

According to Mr Kinnear, three species of Swift occur in Syria, namely *Micropus apus*, Linn., *M. melba*, Linn., and *M. affinis*, Gray and Hardwicke.

Crataerina melbae, Rond.

Chelidomyia melbae, Rondani (1879), *Bull. Soc. Ent. Ital.* xi. 17.

Length, ♂ (one dried specimen) 6 mm., ♀ (one dried and gravid specimen) 7·4 mm.; width of head, ♂ 1·8 mm., ♀ 2 mm.; length of wing, ♂ 7·7 mm.; greatest width of wing, ♂ 2 mm.

In size and general appearance this species superficially resembles *C. propinqua*, Austen, and *C. longipennis*, Austen, having wings considerably longer than the body, with rather less than the distal third strongly attenuate and tapering to a narrow tip. In other respects, however, chiefly as regards the shape of the hind margin of the proximal half, and the condition of the second basal cell, the wing of *C. melbae* presents noteworthy differences from that of either of the species mentioned. The proximal half of the wing of *C. melbae* is relatively broad, the hind margin being convex fully so far as the point at which $M_3 + Cu_1$ (fifth longitudinal vein) would join it if produced; while the second basal cell, which is small, is normally bisected by a supernumerary transverse vein.

Of this species, which is parasitic on the Alpine Swift (*Micropus melba*, L.), the National Collection possesses a ♂ from Switzerland (Fribourg), 5. vi. 1912 (*ex coll.* the late Hon. N. C. Rothschild), and a very old and much damaged ♀, without locality label or other data. The latter specimen exhibits two shining

plates of russet-coloured chitin in front of the apical plates; of these anterior plates, the foremost is very small and triangular in outline, while the other is considerably larger and reniform. The same example shows an abnormality in the right wing, since the dividing vein in the second basal cell, although fully developed in the left wing, is here reduced to a mere vestige.

Genus **Brachypteromyia**, Williston.

Williston (1896), *Entomological News* (Philadelphia), VII. 184.

Genotype: *Anapera fimbriata*, Waterh.

So far as it is possible to judge from the typical specimen (a dried ♀) of the genotype, which is the only species at present known, the *flagellum of the antenna* in this genus is lamelliform, its distal portion broadening out beneath the antennary prolongation into a semihyaline vertical blade, with a bluntly rounded tip. The *wings* in *Brachypteromyia*, which are reduced to the condition of tiny, squamiform processes, exhibit the vestiges of several longitudinal veins, including the costa and two veins behind it, and the proximal portions of two much finer veins, which appear to spring from the hindmost of the former and pass out into the disc. The *femora*, especially those of the first and second pairs of legs, are strongly swollen.

Brachypteromyia fimbriata, Waterh.

Anapera fimbriata, Waterhouse (1887), *Proc. Zool. Soc. London*, 1887, p. 164, and Fig. p. 163.

Brachypteromyia femorata, Williston (1896), *t. cit.* p. 185[1].

B. fimbriata, a North American species parasitic in the Western United States on the White-Throated Swift (*Aëronautes melanoleucus*, Baird), measures some 7 mm. in length, so far as can be judged from the type. According to Dr R. W. Shufeldt, by whom the type was collected, out of fifteen specimens of the Swift mentioned, shot by him at Fort Wingate, New Mexico, 8, 11. ix. 1886, two of the birds each harboured a pair of this parasite[2]. The type of *B. femorata*, Will., was obtained in Wyoming, likewise on *Aëronautes melanoleucus*.

Genus **Myophthiria**, Rondani.

Rondani (1875), *Ann. Mus. Civ. Stor. Nat. Genova*, VII. 464, and (1878) XII. 153 (*Myiophthiria*).

Genotype: *Myophthiria reduvioides*, Rond.

The few species of this genus at present known, to the number of which no additions have been made for nearly fifty years, occur in the Oriental and Australian Regions, where, so far as is shown by the scanty data available,

[1] The identity of *Brachypteromyia femorata*, Will., with *Anapera fimbriata*, Waterh., first suggested by Speiser (1899, *Wien. Ent. Z.* XVIII. 202, note *) and confirmed by Aldrich (1923, *Insec. Inscit. Menstr.* XI. 78), is beyond doubt.

[2] Cf. Shufeldt, R. W. (1887), *The Ibis*, V. 157

they are parasitic upon species of Edible Swift (genus *Collocalia*). Whether or not the condition can be correlated with the nesting of these birds in caves, the fact remains that in *Myophthiria* the *eyes* are very much smaller than in any other genus dealt with in the present paper.

In addition to the genotype, to which further reference is made below, the known species of *Myophthiria* are: *M. capsoides*, Rond. (1878, *Ann. Mus. Civ. Stor. Nat. Genova*, XII. 154), the typical series of which was obtained in the Philippine Is. (*C. Semper*); and *M. lygaeoides*, Rond. (with three instead of two longitudinal veins behind the costa[1]—*ibid.* p. 155), of which two specimens were collected by *Dr O. Beccari*, in Amboina. The name of the host has not been recorded in the case of either of these species, both of which are as yet unrepresented in the British Museum. The latter, however, possesses two ♂♂ of *Myophthiria*, specifically distinct from each other, which cannot be referred to species already described. Of the specimens in question, which, in the absence of further material, it has been thought inadvisable to treat as types of new species, one was obtained at Rockingham Bay, Queensland, nearly eighty years ago (*J. Macgillivray*, H.M.S. "Rattlesnake"); the other was taken at Pundaluoya, Ceylon, January, 1898 (*E. E. Green*), from the nest of a Nilghiri Swift (*Collocalia unicolor unicolor*, Jerdon).

Myophthiria reduvioides, Rond.

Myophthiria reduvioides, Rondani (1875), *Ann. Mus. Civ. Stor. Nat. Genova*, VII. 464, and (1878) XII. 154 (*Myiophthiria*).

The typical series of this species was obtained in Sarawak, Borneo, between 1865 and 1868, by Dr O. Beccari, but the name of the host was not preserved. Subsequently (1878, *Ann. Mus. Civ. Stor. Nat. Genova*, XII. 155), on the authority of von Röder, Rondani recorded the finding of *M. reduvioides* in Fiji, on "*Collocalia vanikorensis*" (= *C. fuciphaga vanikorensis*, Quoy and Gaimard), and "*Emballonura fuliginosa*" (= *E. semicaudata*, Peale). Since the latter is a Bat, it is unlikely, except fortuitously, to have been the host of an avian parasite, even though Bat and Swift inhabit the same cave.

The National Collection possesses a ♂ and ♀ of *M. reduvioides*, measuring respectively 4·75 mm. and 6·25 mm. in length, taken on a Grey-Rumped Swift (*Collocalia francica assimilis*, Stresemann) from a cave near Suva, Fiji, in February, 1884 (*C. M. Woodford*).

[1] In the event of this character being considered of generic value, Rondani proposes the genus *Myiocoryza* for *Myophthiria lygaeoides*.

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HERPETOMONAS IN THE CLOACA OF AFRICAN CHAMAELEONS.

By H. P. BAYON, M.D.

(With a Diagram.)

In a paper read before the Royal Society of South Africa in September 1914, and published in 1915, I gave a brief description of a *Herpetomonas* that was found in great numbers in the cloacal mucus of *Chamaeleon pumilus* on Robben Island, South Africa. The flagellates were present in various stages of development in all the chamaeleons examined, *i.e.* about a dozen, at different seasons of the year. Attempts to cultivate the flagellates on blood agar failed owing to bacterial contamination of the media, and none were discovered in smear preparations of the lizards' spleen and liver or in blood films. Having found *Herpetomonas* in certain flies (*Scatophaga hottentota*) at that time, and it being well known that chamaeleons feed on insects, I concluded that the flagellates present in the lizard were probably derived from insects.

A similar *Herpetomonas* was subsequently found in the cloaca of an undetermined species of lizard, and recently I have discovered flagellates of the same genus in the cloaca of *Chamaeleon dilepis* and *C. senegalensis* at the Zoological Society's Gardens, London. I propose to name the flagellate found in *C. pumilus*, after the late Sir Patrick Manson: *Herpetomonas mansoni*, notwithstanding that Wenyon has described a similar species, *Leishmania chamaeleonis*, found by him in a like habitat in *C. vulgaris*.

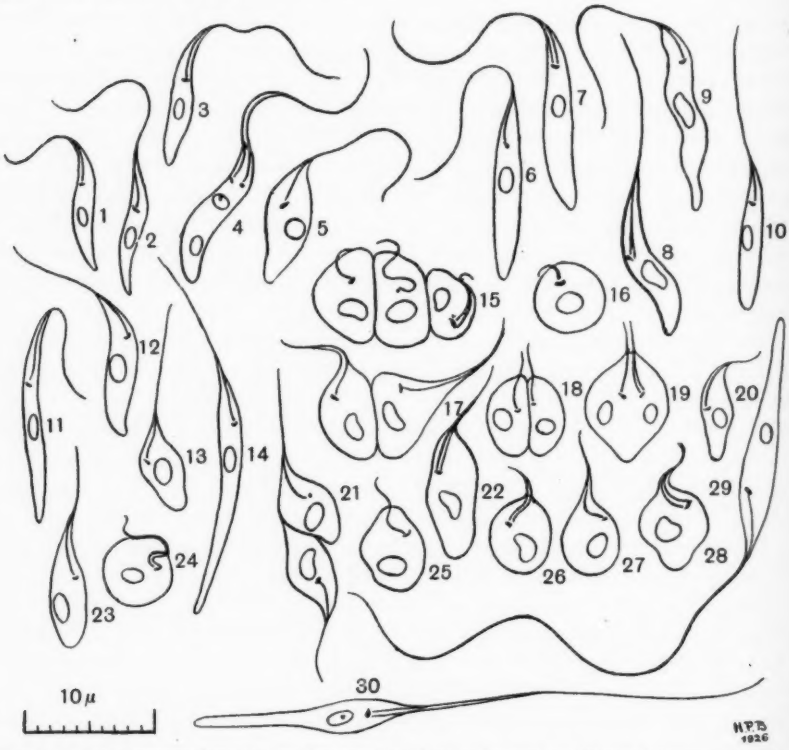
Morphology. The *Herpetomonas* from *C. pumilus* is illustrated in Figs. 1–7, the outlines having been drawn from the original specimens prepared at Robben Island. The scale accompanying the figures indicates the size which was wrongly stated in my first paper (1915) as about three times larger owing to an error in reading the scale of an eye-piece micrometer. The *Herpetomonas* from *C. dilepis* are outlined in Figs. 8–14, and those from *C. senegalensis* in Figs. 15–28. These figures (1–28) speak for themselves, they show thin, thick, and various Leishmaniform flagellates.

For purposes of comparison, *Herpetomonas calliphorae* (Fig. 29) and *H. jaculum* (Fig. 30) are likewise illustrated, these having been drawn from preparations made by Mr C. A. Hoare and Miss M. Vincent respectively.

Movements. When viewed alive by means of dark ground illumination, the *Herpetomonas* exhibit two kinds of movement: (*a*) violent lashing, the body vibrating sideways over the arc of a circle whose centre corresponds about to that of the nucleus of the flagellate; (*b*) speedy progression accompanied by rapid axial rotation, the flagellum acting somewhat after the manner of a tractor screw and the body being twisted on its axis; the flagellates then appearing straight, and the contours hazy whilst they dart across the field of the microscope.

Conclusion.

The presence of *Herpetomonas* in great number in the cloaca of all chamaeleons examined by me and their occurrence in these lizards in widely separated parts of Africa, indicate that they are true parasites of chamaeleons. The manner in which chamaeleons become infected and the life-history of the parasites remain to be determined.



Figs. 1–7. *Herpetomonas* from *Chamaeleon pumilus* (fixation by osmium tetroxyde fumes; absolute alcohol. Stain: Giemsa).

Figs. 8–14. *Herpetomonas* from *Chamaeleon dilepis* (dry fixation. Stain: Leishman).

Figs. 15–28. *Herpetomonas* from *Chamaeleon senegalensis* (dry fixation. Stain: Leishman. Figs. 15–21 were isolated by diluting the cloacal mucus with 1 per cent. NaCl solution).

Fig. 29. *Herpetomonas calliphorae* (wet fixation, Schaudinn. Stain: Iron haematoxylin).

Fig. 30. *Herpetomonas jaculum* (wet fixation, sublimate alcohol. Stain: Iron haematoxylin).

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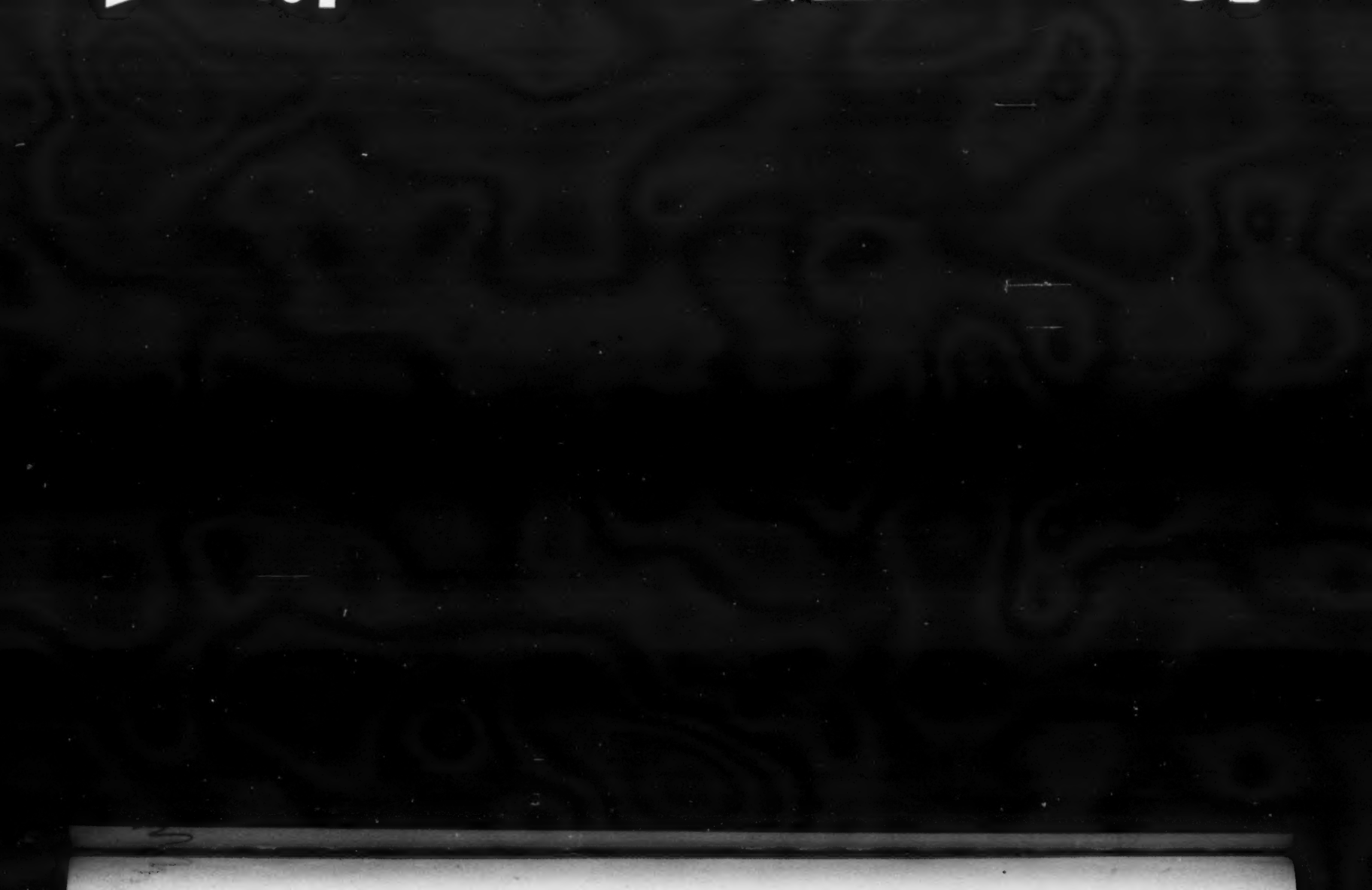
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